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Physiochemical Properties and Bioactive Compounds of Miracle Tree *Moringa oleifera* around Dharan, Sunsari, Province- 01, Nepal

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Abstract
Moringa oleifera Lam. is known by an array of names like ben oil tree, drumstick tree, horseradish tree etc. It is a multipurpose tree having nutritional, medicinal and socioeconomic connotations. It is affluent in healthy bioactive plant compounds. The present study was carried out to investigate the possible bioactive components and examine the physicochemical properties of the ethanol extract of leaves of Moringa oleifera which were extracted using the soxhlet extraction method. Thirteen chemical constituents were identified in the leaf extract. From the qualitative investigation several bioactive components like Flavonoids, Alkaloids, Tannins, Phenolic compounds, Quinine, Anthraquinones, Coumarin, Tannin, Fixed oil, and fats, Steroid-glycosides and Saponins were detected. Specific physicochemical properties of Moringa leaves were examined quantitatively. Extractions were done with ethanol and distilled water as solvents. Standard methods and chemicals were used for the identification of phytochemicals. A good quantity of flavonoids, phenolic compounds, Saponins, Alkaloids, Caumarines, tannins, and steroid-glycosides were found in the leaves of Moringa oleifera but Proteins, Anthroquinones, fixed oil, and fats were not found. Similarly, the physicochemical properties were also investigated. Moisture content was 0.06%, total ash value was 92.88%, the acid value was 5.23, and the extract was highly soluble in ethanol, methanol, diethyl ether, and water but insoluble in chloroform. The medicinal efficiency of Moringa oleifera is due to the occurrence of diverse phytochemical components in leaves. Moringa oleifera leaves may be used as a drug against different ailments for health benefits.

Key Words: *leaves, biological, extract, medicinal, phytochemicals, pharmacological.*

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I. Introduction

Moringa oleifera Lam. is an angiospermous plant, that belongs to the family Moringaceae. *Moringa oleifera* is an aboriginal species to northern foothills of India, Pakistan and Nepal (Leone et al., 2015). Much genetic diversity is mainly available in the Tarai region of Nepal and Uttar Pradesh of India (Ray et al., 2020). The family moringaceae has only one genus called Moringa, with 14 species from tropical and sub-tropical climates (Iqbal & Bhanger, 2006). The *Moringa oleifera* and *Moringa peregrina* are the most common and popular, between these two, the most extensively cultivated species is *Moringa oleifera*. The geographical distribution of Moringa in Nepal varies from tropical to non-tropical regions. *Moringa oleifera* is adapted to a broad array of environmental conditions like warm, humid, and damp conditions. The leaves, bark, flowers, seeds, roots and saps are broadly used in conventional medication, and the leaves, as well as infantile seed pods, are used as foodstuffs in human nutrition (Stohs & Hartman, 2015). Therefore it is typically grown for its healthy pods, flowers and leaves which can be utilized as food, cosmetic oil, drugs, or forage for cattle. According to (Odee, 1998) *Moringa oleifera* is a perennial tree, having well-developed root system, stem and leaves; It attains the height from five to ten meters. Moringa can be used in many different ways. Researches on *Moringa oleifera* tree have accumulated different information on the use of the plant in human consumption, medicinal uses, animal fodder, water purification, fertilizer, living fence, alley cropping, natural pesticide, fuelwood, and growth hormone for plant (Bashir et al., 2016).

Bioactive compounds are phytochemicals that show certain biological activities and have health benefits to the human being. The high number of bioactive compounds might elucidate the pharmacological properties of MO leaves. Several studies, in vitro and in vivo, have confirmed these pharmacological properties (Leone et al., 2015). They have actions on the cells and tissues in the body of living organisms and promote good health. They are present in small quantities in fruits, vegetables, legumes, oils,, nuts, and whole grains. Bioactive compounds are produced as secondary metabolites that could be isolated or extracted from plants. Phytochemicals are found in plants naturally. They induce colour and organoleptic properties in the plants.

Phytochemicals are also referred to as those chemicals that may have biological implications but are not established as essential nutrients in plants (Matos et al., 2015). They could be used as bioactive compounds for straight use as a drug or herbal extracts as botanical drugs. They could be available as nutritional supplements but the prospective health benefits of phytochemicals are derived from the utilization of the whole plant (Rao & Rao, 2007).

Many studies have demonstrated the important effects of *Moringa oleifera* in humans. The plant has been recognized as containing an enormous number of bioactive compounds (Martin et al., 2013). *Moringa Oleifera* leaves are the most extensively studied and they have shown to be beneficial in numerous chronic conditions, as well as hypercholesterolemia, high blood pressure, diabetes, insulin resistance, non-alcoholic liver disease, cancer and overall inflammation (Jimenez et al., 2017). Generally, leaves of Moringa are mainly used in many countries because they are rich in Vitamins, tannins, Carotenoids, Flavonoids, alkaloids, phenolic acids, polyphenols, glucosinolates, isothiocyanates, and saponins (Leone et al., 2015). Moringa contains almost 92 nutrients and 46 types of antioxidants. MO is believed to cure about three hundred diseases and probably have all the vitamins found in fruits and vegetables to a larger extent (Leone et al., 2015).

The leaves are exceptional source of vitamins-A, B when raw as a source of vitamin C. They are also good sources of vitamin D and are among the best plant sources of minerals (Talhaliani & Kar, 2000). The leaves of Moringa are regularly used for remedial purposes as well as for human nutrition because they are rich in antioxidants and additional nutrients, which are commonly deficient in people living in underdeveloped countries (Popoola & Obembe, 2013). MO leaves have been used for the treatment of various diseases from malaria and typhoid fever to hypertension and diabetes (Sivasankari et al., 2014). Leaves of this plant are reported to possess various biological activities, including hypocholesterolemic, antidiabetic, hypertensive agent and (Kar et al., 2003, Faizi et al., 1995), regulate thyroid hormone (Tahiliani & Kar, 2000), central nervous system, digestive system, nutrition and metabolism eye, ear nose throat genito-urinary system (Nadkarni, 2007), to treat gastric ulcers (Pal & Mukherjee, 1995) and scurvy (Selvakumar & Natrajan, 2008).

The leaves, roots, fruits, gum, bark, seed, and seed oil of the plant are reported to have diverse biological activities, including protection against gastric ulcers (Pal et al., 1995), anti-diabetic (Oyedepo et al., 2013), hypotensive (Faizi et al.,1998), and anti-inflammatory effects (Rao & Mishra, 1993). It is also helpful in improving hepatic and renal functions (Bennet et al., 2003), and the regulation of thyroid hormone status (Tahiliani & Kar, 2000). Moringa leaves also protect against oxidative stress (Anwar et al., 2007), inflammation (Mahajan et al., 2009), hepatic fibrosis (Hamza, 2010), liver damage (Pari & Kumar, 2002), bacterial activity (Walter et al., 2011), cancer (Anwar et al., 2007), liver injury (Efiong et al., 2013), and hypercholesterolemia (Halaby et al., 2013).

II. Materials and Methods

Collection of Plant Material

The leaves of *Moringa oleifera* were collected from Vijaypur, Dharan-14 in January 2021. The taxonomic identification of the plant was done at Post Graduate Campus, Biratnagar. The leaves were collected from the plant which was vigorous, healthy and uninfected. The leaves were washed thoroughly under the running tap water to exterminate dust and other foreign particles. After subsequent cleansing, the leaves were shade dried.

Preparation of Leaf Extract

The fresh leaves of *Moringa oleifera* were shade dried. The dried leaves were grounded into powder using the electric grinder. The leaf powder was refluxed with ethanol for 2-3 hours using soxhlet apparatus and the solvent extract was collected in a beaker. The Ethanoic extract was kept in airtight containers and was stored in the freezer (4-20℃) and was used for further qualitative identification of bioactive components and study of physicochemical properties.

Investigation of Physicochemical Properties

Plant leaf powder and ethanol extract were subjected for determining the physicochemical parameters like moisture content, ash values, solubility, pH value in 1% and 10% solution, ethanol extractive values were carried out according to the methods recommended by the World Health Organization (WHO, 1998).

Leaf powder of *Moringa oleifera* was used for the determination of physicochemical parameters such as loss on drying, ash volumes, pH value in 1%solution, ethanol, extractive values were carried out according to the methods recommended by the world health organization.

Determination of Moisture Content

For estimation of loss of moisture on drying leaves, the powder was dried at 105 ℃ for 3 hours in an oven, cooled for 30 minutes in the desiccator and weighed immediately. The loss of weight was calculated as the content of moisture present in air-dried plant material.

Determination of pH range

The Ph value of the extract in 1% W/V (1g: 100ml) of water-soluble portions of the leaf powder of the plant was determined by using a standard simple glass electrode pH meter (Chaudhary & Singh, 2011).

Determination of Total Ash

For the determination of total ash, 2 grams of plant powder of *Moringa oleifera* was placed in a previously ignited (350 ℃ for 1 hour) and a tarred crucible accurately weighed. The dried plant material was spread evenly in the crucible and the material was ignited by gradually increasing the heat to 500 ℃ for 5 hours in a muffle furnace until it is white, indicating the absence of carbon. Cooled and weighed. Total ash content was calculated in mg per g of air-dried material (Belay & Sisay, 2014).

Solubility

The sample was dissolved in different solvents like ethanol, distilled water, and diethyl ether to check the solubility of the leaf extract.

Determination of Acid Value

For the determination of the acid value, 25 ml of ethanol extract was taken in a conical flask and 3 drops of phenolphthalein were added then titrated with 0.1 N KOH (endpoint dark pink colour), then the volume of 0.1 N KOH consumed was noted (Belay & Sisay, 2014).

Acid value= 56.1 x N x V/M

Where, N= Normality of KOH,

M= Mass of sample used

V= Volume of 0.1 N KOH used for titration.

Phytochemical screening

The qualitative analysis of phytochemicals from the ethanol extract of *Moringa oleifera* was individually performed using different standard qualitative tests of Flavonoids, Quinines, Anthraquinones, Saponins, Tannins, Phenolic compound, Steroidglycosids, Proteins, Coumarins, Fixed oil and fats Phytochemical tests were carried out on the ethanol extract following standard methods as described by (Trease & Evans, 1989; Belay & Sisay, 2014).

Test for Flavonoids

To 2 ml of the extract, a few drops of dilute sodium hydroxide were added. An intense yellow colour was produced in the plant extract, which became colourless with the addition of a few drops of dilute acid indicating the presence of flavonoids.

Test for Phenolic Compounds

6 Drops of 1% ferric chloride ($FeCl_3$) solution were added into 5 ml portions of the leaf extract. The appearance of deep violet colour with ferric ions indicating the presence of phenolic compounds was observed (Belay & Sisay, 2014).

Test for Quinines

To determine the presence of quinine in the plant sample, sodium hydroxide was added to the extract. Formation of green colour was obtained indicating the presence of quinines.

Test for Anthraquinones

To investigate the anthraquinone derivatives a specimen was prepared in potassium hydroxide solution, Anthraquinone gives a blood-red colour but the yellow colour was obtained indicating the absence of the compound.

Test for Alkaloids

Mayer's test: 1ml of portions of the extract was acidified with 3 drops of 1 M HCl acid and treated with 5 drops of Mayor's reagent (Potassium mercuric iodide) formation of a yellow or white coloured precipitate or turbidity indicated the presence of Alkaloids (Evans, 2009).

Test for Coumarins

Leaf extract formed yellow colour with 1% KOH in absolute ethanol. 2 ml of portions of 1% solutions of each in test tubes was treated with 7-8 drops of 1 % KOH in absolute ethanol (Belay & Sisay, 2014).

Test for proteins

Xanthoproteic Test – The leaf extracts were treated with few drops of concentrated Nitric Acid in a test tube (Belay & Sisay, 2014).

Test for Saponins

Froth Test: The powdered sample of leaves (2g) were boiled with 8ml of distilled H_2O and filtered. The filtrate when mixed with distilled water, frothing was noticed. The foam produced persisted for more than ten minutes indicating the presence of saponins (Evans, 2009).

Test for Tannins
Ferric chloride test: 4 ml of leaves extract was boiled gently for 5 min and was allowed to cool. Few (5-6) drops of Ferric Chloride solution were added to each extract. Formation of green blackish colour was obtained indicating the presence of tannins in the sample (Evans, 2009).

Test for Steroids
Salkowski Test- Few (5-10) drops of con. H_2SO_4 were added to 2 ml of leaf extract in a test tube. Dark green colouration was noticed indicating the presence of steroids.

Test for Fixed Oils and Fats
Spot test- A drop of the concentrated extract was pressed in between two filter papers and kept undisturbed. Oil stain on the paper was observed indicating the presence of oils and fats in the sample.

Test for Glycosides
For testing glycosides, 5 ml of leaves extract was taken in a test tube and mixed with 25 ml of glacial acetic acid and then 5 ml of conc. sulphuric was added. A reddish-brown colour at the junction of two layers and bluish colour at the upper surface was observed.

Test for Reducing Sugars
Fehling test- Plant extract (2 ml) was taken in a test tube and was mixed with 4 ml of water and 5-10 drops of Fehling's solution were added and heated on the water bath. Brick red precipitate was obtained indicating the presence of reducing sugars in the test sample (Evans, 2009).

III. Results and Discussion

The physicochemical properties were determined quantitatively. The moisture content was 0.06%, pH value was recorded as 5.88, total ash value was 92.88, and acid value was found to be 3.14. Similarly the solubility of the plant material was also determined and found that, it is highly soluble in ethanol, diethyl ether and water but insoluble in chloroform (Table 1). Bioactive components of the *Moringa oleifera* leaves were identified qualitatively (Table 2).

Table 1: Physico-chemical parameters of *Moringa oleifera*

Parameters	**Leaves of *Moringa oleifera***
Moisture content	0.06%
pH range	5.88
Total ash value	92.88
Acid value	3.14
Solubility	Soluble in- Water, Ethanol and Diethyl Ether Insoluble in- Chloroform

Table 2: Phytochemical screening for ethanol extracts of *Moringa oleifera*

Components	**Ethanol extracts**
Alkaloids	++
Anthraquinones	-
Coumarins	++
Fixed oil and fats	-
Flavonoids	+++
Glycosides	+++
Phenolic compounds	+++
Protein	-
Quinine	++
Reducing sugars	+
Steroids	++
Saponins	+
Tannins	++

Note: (+++) good amount, (++) moderate amount, (+) trace amount, (-) absence of constituent.

Bioactive components like alkaloids, phenolic compounds, flavonoids, coumarins, tannins, saponins, steroids, quinines, glycosides, and reducing sugars were found in the leaf sample. Fixed oils and fats, proteins and anthraquinones were not detected in the leaf samples of *Moringa oleifera*. The findings of this study agree with previous studies which also found that, not all phytochemicals are present in all plant parts and that those present also differ according to the type of extracting solvent used (Tijjan et al., 2009; Ayinde et al., 2007). Thus, the presence of such biologically active phytochemical constituents is solely responsible for the medicinal

properties of MO. These components also promote rapid healing and the formation of new tissues in the body of animals (Belay & Sisay, 2014).

The phytochemical screening results showed that Flavonoids were more in amount than any other phytochemicals tested. Flavonoids are strong antioxidants, therefore they are very important. They are also active in reducing high blood pressure (Ayinde et al., 2007). According to some researches, these may modify allergens, viruses, and carcinogens thereby acting as a biological response modifier and acting on bacteria by inhibiting its protein synthesis. Besides this, some in-vitro studies showed that flavonoids could also possess antimicrobial (Galeotti et al., 2008), anti-allergic and anti-inflammatory properties (Yamamato & Gaynor, 2000).

Some phytochemicals like steroid-glycosides, tannins, saponins and coumarins were found to be comparatively small in concentration. The production of sterols is affected by the moisture content in the air. A lower amount of sterols are produced if the moisture content (relative humidity) in the air is high (Bakker, 1991). Steroids are helpful in bone marrow stimulation and its growth. It plays a significant role in preventing bone loss in elderly men (De-piccolli et al., 1991). Alkaloids are nitrogen atoms containing bioactive compounds that occur naturally in plants. They are used in medicine; they have antibacterial as well as antimalarial properties. They also have pharmacological properties, thus they are used as local stimulants and anaesthetic agents. Saponins belong to the class of glycosides, they possess antioxidant, anti-inflammatory and immunostimulant properties so, they are used as an adjuvant in the production of vaccines. Tannins could be the effective ameliorative agent of the kidney. They are used in the treatment of minor burns and ulcers. They are potential anti-viral, anti-bacterial and anti-parasitic agents (Liu, R., 2004). Coumarins belong to benzopyrone chemical class and are found naturally in some plants. They have antiviral, antibiotic, anticancer, anti-coagulant, ant-inflammatory, antifungal, and antioxidant effect (Belay & Sisay, 2014).

IV. Conclusion

In the present study, the leaves of *Moringa oleifera* were investigated thoroughly for their physicochemical characters and chief bioactive components for the analysis of quality, consistency and efficacy for their safe use. The information generated from the study will be helpful in the correct classification and verification of the miracle tree *Moringa oleifera*. The present study showed that the leaf of Moringa oleifera has pharmacologically important chemical compounds such as Alkaloids, glycosides, Saponins, Steroids, Phenolic compounds, Flavonoids, Tannins, Coumarins, and Quinines.

The presence of such bioactive compounds indicates the possible preventive and curative properties of leaves of *Moringa oleifera*. They can be used to treat common medical conditions as well as malnutrition. The leaves can be used in the treatment of common cold, fever, dental problems, oedema, diarrhoea, and flatulence. There is an increasing awareness that many components of traditional medicine are beneficial while others are harmful, hence WHO encourages and supports countries to identify and provide safe and effective remedies, for use in public and private health services (Sofowora, 1993).

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