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INTEGRATED PHYTOCHEMICAL PROFILING AND BIOLOGICAL ACTIVITY ASSESSMENT OF THE METHANOLIC LEAF EXTRACT OF *MORINGA OLEIFERA*

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Abstract

The current work is focused on providing qualitative analysis of plant named Moringa Oleifera. The investigations not only focus on primary data but also on elucidation of secondary data . The aim of the study is to explore the overview of herbal science Operations, Phytochemistry, and medical investigation. In addition, the chemical analysis and antimicrobial effects of Moringa oleifera were investigated using established methodologies. The leaves were shade-dried and subsequently extracted with methanol. The plant component extracts are evaporated and stored in desiccators for further evaluation. The antibacterial properties of Drumstick Tree (Moringa oleifera) were assessed in vitro using the agar disc diffusion method against several pathogenic human microorganisms. The antibacterial potential of Moringa oleifera extracts were tested against E. coli and Staphylococcus aureus. Standard reference antibiotics, doxycycline, and DMSO were employed. The identification of phytochemicals acknowledged the evaluation of secondary metabolites such as flavonoids, saponins, phenols and tannins, proteins and, sugars. Moringa leaves characterization was also analyzed in concentrated form, UV-Visible indicated multiple peaks, measuring the absorption, with UV peaks detected at

229.4885 and 348.7212, while visible peaks were observed at 663.8107 for the extract. In diluted form, absorption was measured, with a UV peak at 226.9821 and visible region peaks at 533.1202 and 660.8269. Moreover, the studies have shown Drumstick trees exhibit various natural functions, such as epidermis lightening and help in regulating the immune system. The pharmacology and phytochemistry of the leaves serve as traditional as well as modern purposes such as food and medicine. Therefore, Moringa plant is like an organic medicine which has several health benefits.

Introduction

Moringa oleifera, widely known by the name as drumstick tree, is a versatile and highly valued plant that has been cultivated for centuries for its nutritional and pharmacological activities. It is included in the family of *Moringaceae* and is widely known in Indian subcontinent [1]. *Moringa oleifera* is widely distributed across various regions of Pakistan, including Balochistan. The drumstick tree for growth prefers a drained soil with pH ranging from 6.4-7.0. On the other side, the plant has wide range of health benefits, that is the reason it is considered as a medicinal plant. In addition, from centuries it has been used by masses in south Asian and African for the treatment of various diseases. It has such chemical properties which could be essential for purification the water. Further, the plant is rich source of vitamins, and minerals and other essential constituents. The Moringa plant has the potential to transform every industry and sector Including; it can be used as food, medicine, and can be used in perfume, soap formation and can also be used to purify [2]. In addition, the plant extract could be helpful against chronic diseases such as cancer and diabetes. The research supports the traditional utilization of plant extracts in folk medicine for various ailments like diarrhea, gastrointestinal issues, throat and ear infections, fever, and skin disorders, along with its potential as a dietary supplement. Overall, *Moringa oleifera* stands as a valuable botanical resource with potent healing properties. On the other hand, the plant extract contains natural products. The Natural products are the most important, large and diversified family representing a variety of bio-activities and has multiple uses in humans, such as in medicinal drugs and in agricultural industry. Furthermore, in medicinal industry natural products are the most save source in discovering new drugs [3] . The natural molecules are classified in two categories large and small natural products. Macro-molecules such as proteins, RNA and DNA they are precursors and intermediates of primary metabolic reactions [4] . The plant has great potential to countering microbes as it contains anti-fungal and anti-bacterial activity. The modern pharmocophores and genomics have potential in improvement to characterize natural products metabolic pathways through bio-informatics drug discovery techniques [5] .

In metabolic reactions **primary metabolites** are released, they are helpful in growth of organism and as well foremost for their evolution. They evolve and play prime role in cell building and other cell functions and are important for survival of organism. Examples include sugars, carbohydrates, nucleotides, amino acids and proteins etc. In brief, current report throws light on primary metabolites role in metabolism and defense against microbes and pathogens [6] .

The **secondary metabolites** are the one most important organic molecule which indirectly play a prime function they are usually produced and released by plants, microbes, and fungi's examples of secondary metabolites include saponins, Terpenoid, alkaloids and flavonoids etc. Moreover, they help in defense and can be used as a shield to protect cell from invasion. This research study was conducted in Quetta, Balochistan. The aim of research is to know the medicinal properties of *Moringa* Plant, Structural elucidation of leaves, general characteristics of *Moringa* Plant, the anti-bacterial activity leaf extract and Phytochemical analysis of leaves of *Moringa oleifera*



Figure 1 Moringa oleifera

Table 1: Classification of Moringa oliefera

Empire	**Plantae**
Sub-Kingdom	*Tracheobionta*
Partition	*Magnoliopsida-Dicotyledons*
Class	*Magnoliopsida*
Genus	*Moringa*
Family	*Moringaceae*

Common Name	*Drumstick tree*
Order	*Brassicales*

Method and Materials

Table 2: list of chemicals apparatus and materials

Chemicals	Apparatus	Materials
Methanol	Measuring cylinder	Lab cot
Dichloromethane	Test tubes	Gloves
Sulfuric acid	Cuvettes	Eyes protection
Copper sulphate solution	Slides	Plant extract
Acetic chloride	Beakers	Grinder
Acetic acid		Filter paper
Sodium Chloride		
Sodium citrate		
Sodium hydroxide solution		

Methodology

Firstly, plant was collected from Loralai and Sibi districts of Balochistan and the experiments were performed in Quetta, then the leaves were cleaned, dried, grinded and then put into methanol. After several days we get methanolic extract of leaves of plant *Moringa oleifera*.

Phytochemical Qualitative Analysis

The techniques for phytochemicals include; Analysis of Saponins (Foam test), Triterpenoids **(**Salkowski test**)**, Tannins (Ferric Chloride test), Steroids, Flavonoids, Analysis of Reducing sugars (Fehling's test), Alkaloids (Wagner test), Phenol test, Steroids test and Analysis of Carbohydrates (Benedict' s test).

Characterization Techniques

UV-VIS spectroscopy:

The UV-VIS spectroscopy method can be used for identification of the functional groups, and conjugated systems. Moreover, the process also quantifies analyte concentration. It works with solid, liquid, and gases.

Evaluation of Phytochemicals

Antibacterial activity:

The methanolic extract of plant resulted efficient anti-bacterial action. Antibacterial activity refers to the ability of a substance or agent to inhibit the growth, reproduction, or kill bacteria. This activity is crucial in combating bacterial infections and preventing the spread of harmful bacteria. This activity was conducted through; Disruption of Cell Wall, Inhibition of Protein Synthesis, and Enzyme Inhibition.

Gram positive:

These bacteria have a thick cell wall made of peptidoglycan. It is a polymer of sugars and amino acids. The thick peptidoglycan layer is responsible for retaining the crystal violet stain during the Gram staining process, giving these bacteria a purple or blue color under a microscope. Examples of Gram-positive bacteria: Staphylococcus

Gram negative:

These bacteria have a thinner cell wall with a more complex structure. The outer membrane of Gram-negative bacteria contains lipopolysaccharides The thin peptidoglycan layer in Gram-negative bacteria does not retain the crystal violet stain during the Gram staining process. Examples of Gram-negative bacteria: Escherichia coli (E. coli).

Results and Discussion

Preparation of plant extract

In the month of November 2022, the plant samples were collected from the Sibi and Loralai districts of Balochistan province. After that plant leaves were washed and cleaned with distilled water, then leaves were left to dry in natural environment. Moreover, after the drying process the leaves were crushed and grinded to form powder.

A solvent extraction technique was employed to extract phytochemicals from the powdered plant sample. The plant material was soaked in methanol, and the resulting mixture underwent rotary

evaporation until a sticky extract was obtained after the evaporation of the solvent. Subsequently, the obtained extract was stored at ambient temperature to facilitate phytochemical and antibacterial analysis.

Phytochemicals qualitative analysis

The phytochemicals can cure diseases without causing any harm to human beings these can also be considered as man friendly medicines.

Flavonoids Test: The methanol crude extract of the plant was combined with 3 ml of ammonia solution, followed by the cautious addition of sulfuric acid. The appearance of a yellow color indicated the presence of flavonoids in the sample. To detect the presence of flavonoids, the methanol crude extract was treated with ammonia solution and sulfuric acid. Flavonoids are a class of plant secondary metabolites known for their diverse range of biological activities and vibrant colors. In this test, the reaction between the flavonoids and the combination of ammonia and sulfuric acid resulted in the formation of colored compounds [7] .

Saponins test:

For the analysis of saponins the methanolic extract was mixed with water. The mixture was shaken for almost 10-15 minutes. The appearance of foam layer indicated the presence of Saponins [8] .

Terpenoids test: The methanolic extract was mixed with 2ml of chloroform and 3ml of concentrated sulfuric acid was added, which in turn show reddish brown color, indicating the presence of terpenoids [9] .

Steroids test: For steroids test analysis the methanolic crude extract sample was mixed with few drops of acetic anhydride and then 2ml of chloroform and sulfuric acid were added. The red color in the solution appeared which indicated the presence of steroids [10] .

Protein test: By mixing methanol extract into beaker containing small amount of copper sulphate and 2ml of sulfuric acid which gives blue tone with positive results [11] .

Glycoside test: In the crude extract of the plant, 2 ml of acetic acid and 2 ml of sulfuric acid were added. The addition of these acids served as a chemical test to detect the presence of glycosides in the sample. The formation of a reddish color in the solution indicated a positive result. The combination of acetic acid and sulfuric acid is commonly used as a test reagent to identify the presence of glycosides in plant extracts. Glycosides are a class of secondary metabolites in plants that are composed of a sugar molecule (glycone) attached to a non-sugar moiety (aglycone). When treated with acids, glycosides undergo hydrolysis, breaking the glycosidic bond and releasing the sugar and aglycone. The reaction between the acids and glycosides results in the formation of various compounds, including colored products, which can be observed visually. In this case, the reddish color formation indicates the presence of glycosides in the crude extract [12] .

Table 3: Results

Phytochemicals	**Tests**	**Reagents**	**Results**
Flavonoids	Flavonoid test	3ml NH_3 soln + 3ml H_2SO_4	Positive
Saponins	• Spot test • Foam test	• Filter paper • 3ml distilled H_2O	Positive
Terpenoids	Salkowski test	3ml CH_2Cl_2 + 2ml H_2SO_4	Positive
Protein	Protein tests	copper-sulphate and sulfuric acid soln	Positive
Steroids	Libermann Bruchard test	2ml CH_2Cl_2 + 2ml H_2SO_4	Positive
Glycoside	Keller Kiliani	3ml H_2SO_4 + CH_3COOH	Positive

UV-visible spectrophotometry

UV-VIS Spectroscopy is the term used for the analytical evaluation of the different types of the solvents and substances. UV-Visible spectroscopic analysis was conducted at room temperature using a Lambda 950 spectrophotometer (Perkin Elmer, USA). The extract and fractions of interest were subjected to

analysis across a wavelength range of 200 to 800 nm. The specific wavelength selected for the study was 300 nm. To prepare the samples, the crude extract was diluted 1:10 with distilled water. Distilled water was used as a blank, and the absorbance of the sample was measured. The process showed peaks at 201.2345 nm and 245.9876 nm in concentrated likewise in visible region one peak was known at 673.7669 nm were resulted. The UV–Vis absorption peaks are also attributed to the presence of alkaloids, phenolic acids, flavonoids, saponins, tannins, terpenoids and carbohydrates in the MO aqueous extract [12] .

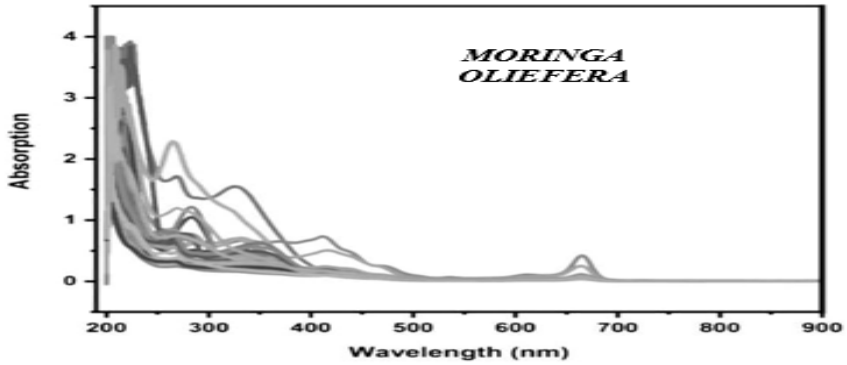


Figure 2 UV visible in concentrated form

In diluted form absorption of methanolic extract was illustrated and measured which showed peak at 240.3254 nm and in visible region peaks are at 436.234 nm and 680.6941 nm was measured.

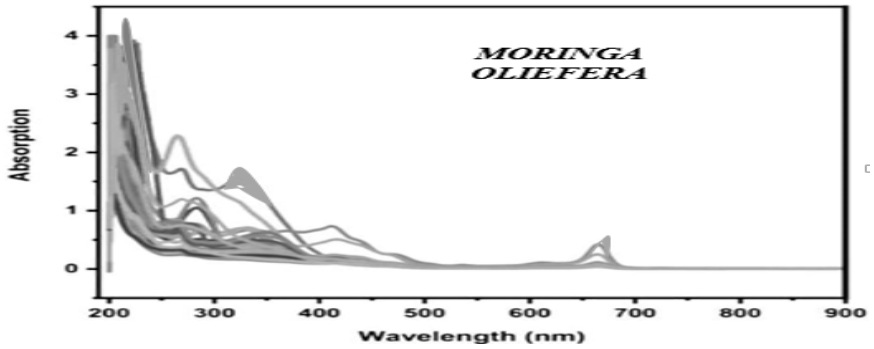


Figure 3 UV Visible in dilute form

Anti-Bacterial Activity

The below outcomes are measured using standard methods and the results indicate the inhibition of bacteria by the methanolic extract of plant. The plant extract shows great results against *Staphylococcus aureus*. ***Moringa oleifera*** can be used as an **antibacterial** agent in human medicine.

The methanolic extract of plant resulted efficient anti-bacterial action.

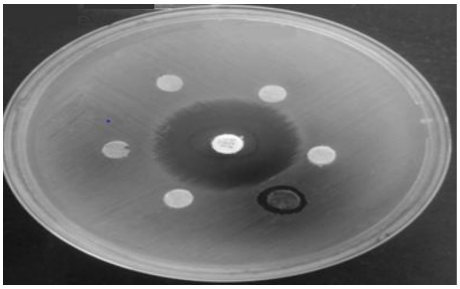


Figure 4 Staphylococcus aureus

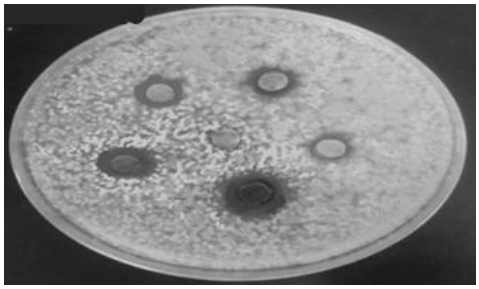


Figure 5 Escherichia coli

Table 4: Concentrations

Extracts and Fractions	Concentration (µg/L)	*E. coli* (µg/L)	*Staphylococcus aureus* (µg/L)
Methanolic Extract	0.1	0	0
	0.12	0	8
	0.14	0	11
	0.16	0	12.5
	0.18	0	16

Discussions:

The researcher [13] elucidated that the *Moringa* plant is full of nutrients and these nutrients can be useful in defense against various diseases. In this research it is found that plant can cure diseases but the amount of plant consumed must be moderate and it effects appears after some days and sometimes for months. The scientist [14] also said that the drumstick tree are beneficial for the growth of human and I also found and agree with the research it has a lot of benefits that can be used in several industries such as food and agriculture industry. Moreover, [15] worked on the leaves of moringa plant and casted that leaves powder of plant can cure cancer and has antimicrobial properties while I also found out that it has anti-bacterial activity against *E.coli* and *staphylococcus aureus*. Furthermore, [16] learn that the seeds and flower of drumstick tree are bioactive with essentials for the body used as food and has usage in pharmacological industry, while I found out that the moringa plant is bioactive. In addition, [17] conducted its research in Lahore, Pakistan and explained that *Moringa* plant is a nutritious plant can be helpful in radical scavenging and contain anti-septic properties and anti-malarial agents and I learn that it has anti septic functions in human body**.** [18] studied that *Moringaceae* family plants are special has medicinal properties and from centuries it has been used as traditional medicine in addition the research also focused on how plant extract fights bacterial and study resulted in it stops bacterial growth and I also find that plant extract can be useful contain anti-oxidant properties with anti-bacterial functions. Further [19] studies the moringa tree and its study aimed at knowing the phytochemicals present in plant extract while the current research outcomes showed that secondary metabolites such as tannins, alkaloids, saponins, terpenoids, phenols and triterpenoids are present in the moringa tree extract. And this phytochemical can be helpful in making several commodity things and could be used in pharmacological industry in making several drugs with variety of uses such as anti-obesity, anti-cancer and anti-malarial and has various health benefits. Further, the Uv-visible detected the alkaloids, saponins, tannins, protein, and carbohydrates in the plant extract, and the findings of [20] were quite similar to results of current study. [21] worked on the pulp and flower of *Moringa oleifera* and illustrated that its extracts fights against bacteria such as *E. coli* and *Staphylococcus aureus.* Current research elucidated that it only kills *Staphylococcus aureus*. This study also found that it has tannins, saponins, phenols and flavonoids and current research also indicated the presence of alkaloids as well.

Conclusion:

The *Moringa Oleifera* is one of the essential natural organic medicinal plants widely utilized in Asian continent. The plant holds immense natural properties. The leaves extract has essential nutrients. The drumstick tree is an organic medicine which got proved by its antimicrobial tests in same study. The methanolic extract showed inhibition zone of 14.2 ± 0.3 mm against E. coli. Further, saponins test illustrates the plant can be useful in cosmetics or soap formation. In addition, it has diverse applications.

The leave extract has demonstrated potential in managing several diseases. For instance, diabetes, cholesterol, colds, sore throats, muscle pain, and hypertension. Additionally, Moringa's bioactive compounds have shown promise in pharmaceutical applications, making it a valuable plant for various purposes. Its crude extract used as for detection of different phytochemical analysis, and showed positive results. *Moringa Oleifera* extract showed characterization i.e. In UV-Visible provides information about the electronic structure and bonding in the molecule. It can be concluded that *Moringa Oleifera* contains various bioactive compounds which makes it an important pharmaceutical plant.

Authors contributions

Collected data and design the experiments; Kamran Ali[1], Shazia Iqbal[1], Asia Ali Mohsin[2], Samra Farooq[3] performed analysis and experiments; Kamran Ali[1], Shazia Iqbal[1], Samra Farooq[3] Inserted data and wrote paper; Kamran Ali[1], Shazia Iqbal[1], Saba Gul[5], Bilal Ahmed[4], Asia Ali Mohsin[2], contributed material and analysis tools; Shazia Iqbal[1], Asia Ali Mohsin[2].

Citation

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