

PHYTOCHEMICAL SCREENING OF POD EXTRACT OF MORINGA OLEIFERA

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ABSTRACT

Phytochemical screening of methanolic extract of pod of Moringa oleifera was carried out by using Soxhlet extractor. The study reveals the presence of various bioactive chemicals. The major phytochemicals identified by screening are Alkaloids, Flavonoids, Protein, Terpenoid. The extraction is carried out by using methanol as a solvent. The Moringa pods were separated and dried separately over ambient temperature further the dried sample will be grinding properly and dried properly and dried powder sample will be extracted in methanol at 65°C by using Soxhlet apparatus and extracts were concentrated by gradually evaporating the respective solvent on rotary evaporator. The concentrated extract was collected in a sterile bottle and kept in a cool and dark place prior to analysis.

INTRODUCTION

Phytochemistry is the study of phytochemicals, which are chemicals derived from plants. Phytochemists strive to describe the structures of the large number of secondary metabolites found in plants, the functions of these compounds in human and plant biology, and the biosynthesis of these compounds. Plants synthesize phytochemicals for many reasons, including to protect themselves against insect attacks and plant diseases. The compounds found in plants are of many kinds, but most can be grouped into four major biosynthetic classes: alkaloids, phenylpropanoids, polyketides, and terpenoids. Phytochemicals or chemicals in plants play important roles in their growth and development. They protect plants from harmful agents such as insects and microbes as well as stressful events such as ultraviolet (UV) irradiation and extreme temperatures.

Phytochemistry is the study of the chemicals produced by plants, particularly the secondary metabolites which are synthesized as a measure for self-defence against insects, pests, pathogens, herbivores, UV exposure and environmental hazards. Phytochemistry takes into account the structural compositions of these metabolites, the biosynthetic pathways, functions, mechanisms of actions in the living systems and its medicinal, industrial, and commercial applications. The proper understanding of phytochemical is essential for drug discovery and for the development of novel therapeutic agents against major diseases. This study introduces phytochemistry, discusses the history of modern phytochemistry, the relationship of phytochemistry with other sciences and the importance of phytochemistry. It also provides information on the sources and classification of phytochemicals, prospects for Phytochemists, the usefulness of computational phytochemistry, biostatistics and the advances in phytochemical research.

The Moringa tree is one of the most incredible plants ever encountered. This may sound sensational, but Moringa's nutritional and medicinal properties have the potential to end malnutrition, starvation, as well as prevent and heal many diseases and maladies worldwide. Moringa is truly a miracle plant, and a divine gift for the nourishing and healing of man. All parts of Moringa tree are edible and have long been consumed by humans. Moringa has many benefits for humans.

Various parts of the Moringa plant are edible. How to use Moringa is also highly variable, ranging from seed or young pods called "drumsticks", very popular in Asia and Africa. In some areas, young pods are most often eaten, while the Moringa leaves are the most commonly used. The flowers are edible when cooked and tasted like mushrooms. Bark, sap, roots, leaves, seeds, oil and flowers are used in traditional medicine in several countries.

Moringa leaves are the most nutritious part of the Moringa plant itself, is an important source of vitamin B6, vitamin C, provitamin A as beta-carotene, magnesium, and protein among other nutrients that have been studied in the laboratory by the USDA. When compared with the general food, Moringa leaves are very high in nutrients and the leaves are traditionally cooked and used like spinach. Besides being used fresh as a substitute for spinach, leaves are dried and ground into a powder to use in soups and sauces. Moringa tree and the leaves have been used to combat malnutrition, especially among infants and nursing mothers.

MATERIAL AND METHOD

Collection of Plant Materials:

The fresh Plant Was collect from The Amaravati City (Maharashtra). Amravati district is a District of Maharashtra State in central India. Amravati is the Administrative Headquarters of the District

Preparation of the Plant Extract:

The plant pods were separate and dried separately over ambient temperature. Further the dried sample will be grinding properly and dried properly and dried powder sample was extracted in methanol at 650C by using Soxhlet apparatus and extracts was concentrated by gradually evaporating the respective solvent on rotary evaporator. The concentrated extract was collected in a sterile bottle and kept in a cool and dark place prior to analysis.

Phytochemical Analysis (qualitative Analysis):

The following Phytochemicals were tested during Phytochemical screening

1. Glycosides
2. Saponins
3. Alkaloids
4. Flavonoids
5. Tannins
6. Proteins
7. Carbohydrates
8. Phytosterols/Terpenoids
9. Phenol

1. Saponins -

Saponins are a varied group of compounds widely distributed in the plant kingdom, which are characterized by their structure containing a triterpene or steroid aglycone. Increasing demand for natural products coupled with their physicochemical that is surfactant properties and mounting evidence on their biological activity such as anti-cholesterol activity and anticancer activity has led to the emergence of saponins as commercially significant compounds with expanding applications in food, cosmetics, and pharmaceutical industry. (Ozlem Guçlu and G Mazza, 2007).

Test for Saponins:

Foam Test: 0.5 gm of extract was shaken with 20 ml distilled water, if foam produce persists for ten minute it indicates the presence of saponins. (Trease, GE and Evans W.C., 2002).

2. Alkaloids -

Alkaloids have very unlike and important physiological effects on humans and other animals. Generally known alkaloids include morphine, ephedrine, strychnine, quinine and nicotine. Alkaloids are found primarily in plants and are especially common in certain families of flowering plants. In fact, as many as one-quarter of higher plants are estimated to contain alkaloids, of which several thousand different types have been identified. Generally, a given species contains only a few kinds of alkaloids, though both the opium poppy (*Papaver somniferum*) and the ergot fungus (*Claviceps*) each contain about 30-32 different types. The function of alkaloids in plants is not yet clear. It has been suggested that they are the waste products of plants metabolic processes, but proofs suggest that they may

serve specific biological functions. In few plants, the concentration of alkaloids increases just before to seed formation and then drops off when the seed is ripe, suggesting that alkaloids may play a role in this process. Alkaloids may also protect some plants from destruction by certain insect species. The chemical structures of alkaloids are extremely variable. Generally, an alkaloid contains at least one nitrogen atom with an amine type structure i.e. one derived from ammonia by replacing hydrogen atoms with hydrogen-carbon groups called hydrocarbons. (Britannica Online Encyclopaedia Alkaloid Chemical Compound, 2020)

Test for Alkaloids:

Dragendorff's Test:

1 ml of the filtrate was treated with few drops of Dragendorff's reagent. Orange brown precipitate indicated the presence of alkaloids.

3. Flavonoids -

Flavonoids are various compounds found naturally in most of the fruits and vegetables. They are also found in plant products like wine, tea, and chocolate. There are six different types of flavonoids found in food, and each kind is broken down by our body in a different way.

Flavonoids are rich in antioxidant activity and can help our body ward off every day toxins. Including more flavonoids in our diet is a great way to help our body stay healthy and potentially decrease the risk of some chronic health conditions. Many plant products contain dietary flavonoids. These types of flavonoids are well established for their antioxidant properties. They may manage symptoms of cardiovascular disease. Flavonoids may regulate cellular activity and fight off free radicals that cause oxidative stress on the body. In simpler terms, they help our body function more efficiently while protecting it against everyday toxins and stressors.

Flavonoids are also powerful antioxidant agents. Antioxidants help our body fight off potentially harmful molecules that can be introduced to the body. Our body produces antioxidants naturally, but they are also found in dark chocolate, legumes, and many fruits and vegetables.

Inflammation is one of the body's immune responses. Allergens, germs, toxins, and other irritants can trigger inflammation which results in uncomfortable symptoms. Flavonoids may help our body dismiss that inflammatory reaction so that those symptoms are minimized.

As there are different flavonoids which can help the body in different ways. For one, including foods with flavonoids in our diet may be an effective way to help manage high blood pressure. At least five sub-types of flavonoids have a notable effect on lowering high blood pressure. Also, the flavonoids found in tea, coffee, and soy may help lower the risk of having a heart attack or stroke.

A diet with high in flavonoids may also decrease the risk of type 2 diabetes. Lower risk of type 2 diabetes can be correlated with high intake of dietary flavonoids. However, more research is needed to prove the ability of flavonoids as blood sugar regulators.

The antioxidant and anti-inflammatory effects of flavonoids have also triggered the idea to new researches to study their efficiency as anticancer drugs. Certain flavonoids may help to stop the cancer cells from multiplying. Including foods with flavonoids and keeping a healthy diet may decrease the risk of getting certain cancers.

Test for Flavonoids:

Ferric Chloride Test: To the extract, a few drops of neutral ferric chloride solution were added, a blackish red colour forms, indicates the presence of flavonoids.

4. Protein:

"Proteios" is a greek word which means primary from which protein word is derived. As the name suggested, the proteins are of paramount importance for biological systems. Out of the total dry body weight, 3/4th is made up of proteins. Proteins are used for body building; all the major structural and functional aspects of the body are carried out by protein molecules. Abnormalities in protein structure will result to molecular diseases with profound alterations in metabolic functions. Proteins contain Carbon, Hydrogen, Oxygen and Nitrogen as the major components, while Sulfur and Phosphorus are minor constituents. Nitrogen is prominent characteristic of proteins. On an average, the nitrogen content of ordinary proteins is 16% by weight. All proteins are polymers of amino acids (Vasudevan et al., 2017).

Test for Protein:

Biuret Test: To the 3 ml of aqueous extract. 10% NaOH and few drops of 0.1% CuSO₄ solution were added. Appearance of violet or pink colour, reveals the presence of proteins.

5. Carbohydrates:

A carbohydrate is a biomolecule consisting of carbon (C), hydrogen (H) and oxygen (O) atoms, usually with a hydrogen-oxygen atom ratio of 2:1 (as in water) and thus with an empirical formula of C_m (H₂O)_n. However, all carbohydrates are not conforming to this precise stoichiometry (e.g. deoxy-sugars such as fructose). Also, not all the chemicals which conform to this definition automatically classified as carbohydrates e.g. formaldehyde.

In the biochemistry, the term is most common, where it is a synonym of saccharide, a group which includes sugars, starch, and cellulose. The saccharides are further divided into four chemical groups namely monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Monosaccharides and disaccharides are the lower molecular weight carbohydrates, and are commonly known as sugars.

Test for Carbohydrates:

Fehling's Test: Take 2 ml of extract in a test tube and add 1-2 ml each of Fehling's solution A and Fehling's solution B into it. Keep the test tube in a boiling water bath for some time. Accordingly, formation of red precipitate confirms the presence of reducing sugar.

6. Terpenoids/Phytosterols:

Phytosterols are also called as isoprenoids, which are the most numerous and structurally very diverse natural products found in many plants. Several studies, in vitro, preclinical, and clinical have confirmed that this class of compounds displays a wide array of very important pharmacological properties. The diverse collection of terpenoid structures and functions have provoked increased interest in their commercial use resulting in some established medical applications being registered as drugs on the market (A. Ludwiczuk, 2017).

Test for Terpenoids/Phytosterols:

Salkowski Test: Take 2 ml of plant extract, and add 2 ml of chloroform and 2 ml of concentrated H₂SO₄ in the same test tube, shake well. Two layers are appeared, chloroform layer is red and acid layer is golden yellow fluorescent. This indicates the presence of phytosterols.

7. Phenols:

Phenolics are aromatic benzene ring compounds with one or more hydroxyl groups produced by plants mainly for protection against stress. The functions of phenolic compounds in plant physiology and interactions with biotic and abiotic environments are difficult to guess. Phenols play very important roles in plant development, particularly in lignin and pigment biosynthesis. They also give the structural integrity and scaffolding support to plants. Beside this, phenolic phytoalexins, secreted by wounded or otherwise perturbed plants, kill or repel many microorganisms, and some pathogens can counteract or nullify these defences. (Amita Bhattacharya, 2010)

Test for Phenols:

Ferric Chloride Test: 5% solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the extract. Appearance of dark green or deep blue colour reveals the presence of phenols.

8. Coumarins:

Coumarins are phenolic, natural volatile active compound found in many plants composed of fused benzene and α -pyrone rings. More than 1300 different coumarins have been identified. Coumarins have antithrombotic, anti-inflammatory, and vasodilatory activities. Warfarin an anti-coagulant drug is the most popular and is used as an oral coagulant and rodenticide. Coumarins may also shows antiviral effects and are highly toxic in rodents.

Test for Coumarins:

0.5 g of the extracts was taken in a test tube. The mouth of the tube was covered with filter paper moistened with 1 N NaOH solution. Test tube was deep for few minutes in boiling water bath and then the filter paper was removed and examined under the UV light. If there is yellow fluorescence, then it indicates the presence of coumarins.

9. Chalcone:

Chalcone is a member of the class of acetophenone in which one of the methyl hydrogens has been replaced by a benzylidene group. It has a role as a plant metabolite. It is a member of styrene's and a member of chalcones. Group of plant-derived polyphenolic compounds belongs to the flavonoid's family. Studies reflected that, some chalcones possess a wide variety of cytoprotective and modulatory functions, which may have therapeutic potential for multiple diseases. The extent of their biological activity is defined by their physicochemical properties.

Test for Chalcone:

2 ml ammonium hydroxide was added to 0.5 ml of the extract. Appearance of reddish-brown colour, reveals the presence of chalcone.

Table No. 1 Phytochemical screening

Sr. No.	phytochemicals	Test performed	observation
1	Saponins	Foam Test	-
2	Alkaloids	Dragendorff's Test	+
3	Flavonoids	Ferric chloride test	+
4	Protein	Biuret test	+
5	Carbohydrates	Fehling test	-
6	Terpenoid	Salkowski's Test	+
7	Phenol	Ferric Test	-
8	Cumarines	Ferric chloride test	-
9	Chalcones		-

The present study the results of preliminary phytochemical screening from the Methanolic extracts of the leaves of the *Moringa Oleifera*. Plant had been found to be rich in phytoconstituents such as alkaloids, phenols, flavonoids, steroids, coumarins.

Foam test for saponins showed negative (-) results for leaves. Hager Test for alkaloids shows positive (+) results for leaves extract. Ferric Chloride Test and Lead acetate solution test for showed positive (+) results for leaves extract.

Conclusion:

Pod of *Moringa Oleifera* were collected and extracted in methanol, evaluated for its phytoconstituents present in them. Then present study provides evidences that solvent extract of *Moringa Oleifera* contains medicinally important bioactive compound and this justifies the use of plant species as traditional medicine for treatment of various diseases.

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