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Phytochemical and biochemical analysis of leaf, pulp and seed extracts of *moringa oleifera,* lamk

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Abstract
Phytochemical analysis of *Moringa oleifera* leaves contain alkaloids, flavonoids, carbohydrates, proteins, tannins and steroids. In pulp carbohydrates, proteins, starch, saponins, terpenoids and glycosides were present. The seeds contain alkaloids, carbohydrates, proteins, starch, tannins, saponins and glycosides. The biochemical analysis showed that total carbohydrate content in the seed of *Moringa oleifera* contain 93.75mg and in protein was 9.6mg and starch is 84.37mg. In *Moringa oleifera* phytochemical studies in leaves revealed alkaloids, flavonoids, steroids, tannins, carbohydrate and proteins were found to be high. In pulp carbohydrates, proteins, starch, saponins, terpenoids and glycosides present. In seeds carbohydrates, proteins, alkaloids, starch, glycosides, saponins and tannins are present.

Keywords: *Moringa oleifera*, Moringaceae, biochemistry, phytochemistry.

Introduction
Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. Phytochemicals defined in the strictest sense, as chemicals produced by plants. However, the term is generally used to describe chemicals from plants that may enhance health status of organisms, but are not essential nutrients. There is ample evidence to support the health benefits of the diet in the form of fruits, vegetables, legumes, whole grain and nuts. Phytochemicals are biological active, naturally occurring chemical compounds found in plants, which provide health benefits for human further than those attributed to macronutrients and micronutrients. They protect plants from disease and damage and contribute to the plants color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, droughts, UV exposure and pathogenic attack are called as phytochemicals.(Amritpal singh saroya., 2011) [1].

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 biochemicals like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life processes. These medicinal plant species are used either as food or food supplements along with their medicinal benefits. (Samidha M Pawaskar *et al*., 2017) [10].

Moringa is native to the Himalayan foothills (India/Bangladesh). As a commercial crop, it is cultivated extensively in India and parts of Africa. It would be challenging to find a region in the tropics or subtropics where *Moringa* is not grown as a backyard tree for leaf and pod consumption, medicinally and for fiber. Moringa is most commonly found in areas with South and Southeast Asian (particularly Filipino) populations. Today it is widely cultivated in Africa, Central and South America, Sri Lanka, India, Mexico, Malaysia, Indonesia and the Philippines. It is considered one of the world's most useful trees, as almost every part of the Moringa tree can be used for food or has some other beneficial property.

The leaves, seeds and flowers have been reported to have good nutritive and medicinal value. The seeds are consumed raw or roasted while the flowers are cooked in soups and resemble mushrooms; the leaves are cooked as vegetables. The flowers and leaves are rich in vitamins A, B1, B2, B6, E and C. They are among the best sources of minerals. The plant has been implicated in the treatment or suppression of many degenerative diseases among many rural consumers. *Moringa* is a good source for essential amino acids especially Lysine and essential minerals such as Na, K, Mg, Ca, P and Fe. (Sobhy and Sohaimy *et al.*, 2015) [12].

Moringa seed cake, obtained as a byproduct of pressing seeds to obtain oil, is used to filter water using flocculation to produce potable water for animal or human consumption. Moringa seed cake removes most impurities from water. This use of particular interest for being nontoxic and sustainable compound to other materials in *Moringa* growing regions where drinking water is affected by pollutants. *Moringa* cures the tired blood (anemia), arthritis, cancer, constipation, birth control, diabetes, diarrhea, stomach pain, head ache, heart problems, high blood pressure, kidney stones, swellings, thyroid disorders, infections, stimulating our immunity.

Leaves of *Moringa* have outstanding nutritional qualities. It is among the best of all perennial vegetables. It is high in β-carotene, amino acids and ascorbic acid. Hence, it is used to increase milk production in lactating mothers and also useful in treating scurvy, respiratory ailments and as emesis remedy. *Moringa oleifera* leaves showed antioxidant activity that was stable in pH 4 and 9; therefore, this plant extract is a potential source of natural dietary antioxidants in food supplements due

to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids. The juice from the leaf of *Moringa* can reduce glucose levels; it has purgative, anti-inflammatory and strong antimalarial properties. It also serves as an antidote to piles, fevers, sore-throat, bronchitis, catarrh, eye and ear infections as well as sore healing and relieve of headaches. (Jikasmita Dalei *et al*., 2016) [4].

Habit of *Moringa oleifera* tree

Fig 1

Material and methods

Study area

In the present study plants are collected from Semmedu, Coimbatore district Tamil Nadu, India. Semmedu has the altitude of 312 meters above the sea level.

Sample collection

Fresh seeds, leaves pulp of the selected plant materials were collected during March. They were cut in to small pieces, shade dried and ground to fine powder and stored in air tight container for further analysis.

Preliminary phytochemical analysis

Preparation of ethanolic extract (15g) with ethanol (70%) in a shaker system for 48hrs.The nature and yield of the extract were noted. The extracts were stored in a refrigerator at 4ºc for further studied. The ethanol extracts of the selected plant materials such as pulp, leaves and seeds were tested for Carbohydrates, Proteins, Starch, Amino acids, Steroids, Glycosides, Flavonoids, Alkaloids, Tannins, Saponins, Terpenoids and Resins. This phytochemical screening of the extracts was carried out by standard methods. (Raaman, 2006; Karpagam et al., 2008; Kokate et al., 2001) [8, 7].

1. Test for carbohydrates

To 2ml of test solution adds two drops of the Molish reagent (a solution of α napthol in 95% ethanol). The solution is then poured slowly into a test tube containing 2ml of conc. sulphuric acid so that two layers form. The formation of a purple product at the interface of the two layers indicated the presence of carbohydrates.

2. **Test for Proteins**

It is used to determine the presence of peptide bonds in protein. To 3 ml of test sample add 3%NaOH and few drops of 1 % $CuSO_4$. The solution turns from blue to violet (purple) or to pink indicated the presence of protein.

3. Test for Starch

Mix 3ml test solution and few drops of dilute iodine solution. Blue color disappears on boiling and reappears on cooling indicated starch.

4. Test for amino acids

To 5ml of test sample solution add a few drops of 40 % NaOH and 10% lead acetate boiled the solution formation of black precipitates indicated presence of amino acid.

5. Test for steroids

To 2ml of extract add 2ml chloroform and 2 ml conc. Sulphuric acid. Shake well, chloroform 1 layer appear red and acid layer show greenish yellow florescence which indicated the presence of steroids.

6. **Test for glycosides**

To the solution of extract add glacial acetic acid, few drops 5% ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and bluish green colour in upper which indicated presence of glycosides.

7. Test for flavonoids

To 2 ml of extract add few drops of 1% Ammonia solution. A yellow coloration was observed for the presence of flavonoids.

8. Test for alkaloids

To 0.5g of each extracts adds 5ml of 1% aqueous hydrochloric acid and kept in water bath: 1ml of the filtrate is to be treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicated the presence of alkaloids.

9. Test for tannins

To 0.5ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour indicated for Gallic tannins and green black for catecholic tannins

10. **Test for Saponins**

To 1ml extract solution, 1ml of water and shake it. Persistent foam indicated presence of saponins.

11. Test for terpenoids

2ml extract was mixed with 2ml chloroform in a test tube. To this 3ml Conc. Sulphuric acid was carefully added along the wall of the tube to form a layer. An interface with a reddish brown coloration indicates the presence of terpenoids.

12. **Test for gums**

To 1 ml of extract add 3ml of dil.Hcl; Feling's solution is added drop by drop till red colouration indicated the presence of gums.

Biochemical analysis

The biochemical analysis is performed on *Moringa oleifera*, Lamk. The powder of selected plant seed where tested for estimation of carbohydrate (Anthrone method), protein (Lowry's method) and starch by (Hedge and Hotreiter method)

Estimation of carbohydrate by Anthrone Method

100mg of dried powdered seed was hydrolysed in a boiling water bath for 30 minutes with 80%ethanol in water and centrifuge 8000g for 15 minutes. And preserved 4ml of supernatant. From it 1ml of the supernatant dried and dissolved in 50 ml distilled H_2O. Anthrone reagent is prepared by mixing 300gm anthrone with 150ml ice cold H_2SO_4. 0.2ml of sample made up to 1ml with distilled H_2O add 4ml of Anthrone reagent and rapidly cooled in ice bath. OD values which was read at 630nm using BSA (Bovine Serum Albumine)

Protein estimation

1gm of the sample weighed and grained well with a pestle and motor in 1ml of the buffer. Add 5% of TCA and kept in cooled for 1hour. Centrifuge at 3500 rpm for 20 minutes. Dissolved precipitated protein in 0.1N NaOH (Reagent A). 0.5% $CuSO_4$ in 1% potassium sodium tartarate (Reagent B). 50ml of reagent A and B was mixed prior to use and reagent C was obtained. Which was immediately added in to the test tube was mixed well and allowed to stand for 10minutes. 0.5ml of reagent D (Folin Ciocalteau reagent) was added, mixed well and incubated at room temperature in the dark for 30 minutes to develop blue colour. OD values read at 660 nm using glucose as standard and calculate the amount of protein (Lowry *et al.*, 1951).

Estimation of starch (Hedge and Hotreiter, 1962)

The total soluble carbohydrates from the selected sample were extract and estimated by the Anthrone reagent method (Hedge and Hotreiter, 1962). Using glucose as standard at 620nm in a spectrophotometer the OD values were expressed as mg/100gm on dry weight basis.

Result

The present study was carried out in *M. oleifera* revealed the presence of alkaloids, flavonoids, carbohydrates, steroids, glycosides, proteins, saponins, tannins and terpenoids in ethanolic extracts as shown in table 1.Phytochemical analysis of *Moringa oleifera* leaves contain alkaloids, flavonoids, carbohydrates, proteins, tannins and steroids. In pulp carbohydrates, proteins, starch, saponins, terpenoids and glycosides were present. The seeds contain alkaloids, carbohydrates, proteins, starch, tannins, saponins and glycosides. Biochemical constituents like carbohydrate, proteins and starch as shown in table 2.The biochemical analysis showed that total carbohydrate content in the seed of *Moringa oleifera* contain 93.75mg and in protein was 9.6mg and starch is 84.37mg.

Table 1: Phytochemical constituents of *Moringa oleifera* seed, pulp and leaf extracts

Phytochemicals	*Moringa* seed	*Moringa* pulp	*Moringa* leaf
Alkaloids	+	-	+
Flavonoids	-	-	+
Carbohydrates	+	+	+
Proteins	+	+	+
Starch	+	+	-
Tannins	+	-	+
Saponins	+	+	-
Terpenoids	-	+	-
Glycosides	+	+	-
Steroids	-	-	+

(+ indicates present and – indicates absent)

Table 2: Carbohydrate, protein and starch content of *Moringa oleifera* seeds

Sample	Carbohydrate (mg)	Protein (mg)	Starch (mg)
Moringa seed	93.75	9.6	84.37

Discussion

In *Moringa oleifera* phytochemical studies in leaves revealed alkaloids, flavonoids, steroids, tannins, carbohydrate and proteins were found to be high. In pulp carbohydrates, proteins, starch, saponins, terpenoids and glycosides present. In seeds carbohydrates, proteins, alkaloids, starch, glycosides, saponins and tannins. The ethanolic extracts of *Moringa oleifera* contain alkaloids, steroids, flavonoids and tannins in similar result were reported by Shikhakhandelwal *et al.*, (2010) whereas Josephine *et al*., (2010) [5] reported that alkaloids were absent for the ethanolic extract of *M.oleifera* and steroids were not detected (Ajayi *et al*., 2015) [2] and protein were absent (Rashasaad *et al.*, 2014) in the ethanolic extract of *M. oleifera.*

Alkaloids are naturally occurring chemical compounds containing pharmacological effects and are used as medications and recreational drugs. Flavonoids enhance the effects of Vitamin C and function as an antioxidant. They are also known to be biologically active against liver toxins, tumor, viruses and other microbes. (Amritpal singhsaroyo, 2011) [1] Glycosides are water soluble constituents found in the cell sap. They are colourless crystalline substances. It is more important in medicine than a lot of drug. It occurs in higher plant tissue in very small amounts and it is used therapeutically (Anna drew 2011). Saponins are glycosides which are components in many traditional and herbal medicines. These are secondary metabolites and it is found in a wide range of plant parts like bark, leaves, stems, roots and even flowers. They have various biological activities including hepatoprotective, anti-ulcer, anti-tumor and anti-microbial (Eskandar *et al.,* 2015) [3]. Tannins are widely distributed in plant flora. Tannins are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissue. The growth of many fungi, yeast, bacteria and viruses was inhibited by tannins (King – Thom *et al*., 1998) [6].

Terpenes protect cells from becoming cancerous, slow cancer cell growt h, strengthen immune function, limit production of cancer –related hormones, fight viruses, and work as antioxidant. Starches are related to sugars in that they are made up of many glucose molecules bonded together. Some starches are resist digestion in our gut. These resistant starches, rather than acting as a nutrient, behave similarly to dietary fiber and can help regulate blood sugar, improve appetite control and promote health. Steroids work by decreasing inflammation and reducing the activity of the immune system. They are used to treat a variety of inflammatory diseases and conditions.

The biochemical analysis showed that total carbohydrate content in the 100g of *Moringa oleifera* seeds contains 93.75mg. Carbohydrate is one of the most important components in many foods. These can only be stored in limited quantities. Carbohydrates are one of the main types of nutrients and the one needed in the largest amounts by the body. The role of carbohydrates is to provide energy, as they are the body's main source of fuel. It's important for intestinal health and waste elimination. Proteins are important constituents of food form a number of different reasons. They are a major source of energy as well as containing essential amino acids such as lysine which are essential to human health (Amritpal Singhsaroya 2011) [1].

Conclusion

The present study proved that the *Moringa* leaves, pulp and seeds were found to be rich in carbohydrate, proteins. *Moringa* is an excellent source of protein, carbohydrate, alkaloids, flavonoids, tannins and steroids. It contain two times the amount of protein of yogurt, four times the amount of vitamin A as carrots, three times the amount of potassium as bananas, four times the amount of calcium as cow's milk. Finally *Moringa oleifera*, Lamk. is consumed in daily diet to improve hair growth, its cure anemia, arthritis, and other joint pain, such as rheumatism, kidney stone, bacterial, fungal, viral and parasitic infections

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