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Biochemical and Nutritional Analysis of the Leaf Extract of *Moringa oleifera* Lam.

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ABSTRACT

The present study was conducted to investigate the presence of biochemical contents viz., proximate and micronutrient analysis in the leaves of Moringa oleifera Lam. The biochemical contents were determined by different biochemical methods. Moringa oleifera Lam. leaves confirmed the presence of all the essential nutrients, minerals and vitamins in good amounts and possess good nutritive value. The plant leaf powders can thus be looked forward as the probable sources of food supplementation in future, after further investigation of the anti-nutritive factors present in them and their enzymatic and molecular effect on human health.

Keywords: *Moringa oleifera* Lam.; Proximate principles; Micronutrients

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INTRODUCTION

The tenet "Let food be thy medicine and medicine be thy food," espoused by Hippocrates nearly 2,500 years ago, is receiving renewed interest. In particular, there has been an explosion of consumer interest in the health enhancing role of specific foods or physiologically-active food components, so-called functional foods [1]. Clearly, all foods are functional, as they provide taste, aroma, or nutritive value. Within the last decade, however, the term functional as it applies to food has adopted a different connotation- that of providing an additional physiological benefit beyond that of meeting basic nutritional needs. The past decade has witnessed intense interest in "nutraceuticals" (or "functional foods") in which phytochemical constituentscan have long-term health promoting or medicinal qualities. Althoughthe distinction between medicinalplants and nutraceuticalscansometimes be vague, a primary characteristic of the latter isthatnutraceuticals have a nutritional role in the diet and thebenefits to health may arise from long-term use as foods (i.e. chemoprevention) [2]. Some of the plants with promising bioactive properties also contain useful minerals and food value for human and animal consumption. 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. Evaluation of the biochemical and nutritional significance of these plants thus can help to understand the worth of these plants species [3]. As far herbal drug's standardization is concerned, WHO also emphasizes on the need and importance of determining proximate and micronutrients analysis. Such herbal formulations must pass through standardization processes [4]. In the present study, the medicinal plants species viz., *Moringa oleifera* Lam. was subjected to proximate and micronutrient analysis. The total carbohydrates, reducing sugars, protein and fat were analyzed so also the micronutrient content i.e. minerals like Ca, P, Fe and Mg levels were analysed, so also both water soluble and water insoluble vitamins were estimated using biochemical methods.

MATERIALS AND METHODS

Analysis of Proximate Principles of Diet

Determination of total carbohydrates by anthrone method:

Carbohydrates are the important components of storage and structural materials in the plants. They exist as free sugars and polysaccharides. The basic units of carbohydrates are the monosaccharides which cannot be split by hydrolysis into more simpler sugars. The carbohydrate content can be measured by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharides [5].

Determination of total reducing sugars by Folin – Wu method [6-8]:

Determination of reducing sugar (glucose) by Nelson-Somogyi method: Sugars with reducing property (arising out of the presence of of a potential aldehyde or keto group) are called reducing sugars. Some of the reducing sugars are glucose, galactose, lactose and maltose. The Nelson-Somogyimethid is one of the classical and widely used methods for the quantative determination of reducing sugar especially glucose [5].

Estimation of cellulose content:

Cellulose, a major structural polysaccharide in plants, is the most abundant organic compound in nature, and is composed of glucose units joined together (β (1→4) glycosidic linkage) in the form of repeating units of the disaccharides cellobiose with numerous cross linkages [5].

Estimation of crude fibre content:

Crude fibre consist largely of cellulose and lignin (97%) plus some mineral matter. It represents only 60% to 80% of the cellulose and 4% to 6% of the lignin. The crude fibre content is commomnly used as a measure of the nutritive value of livestock feeds and also in the analysis of various foods and food products to detect adulteration, quality and quantity [5].

Estimation of total protein content by folin lowry's method [5,9]:

Proteins can be estimated by different methods as described by Lowry et al. [10] and also by estimating the total nitrogen content (micro-kjeldahl method). No method is 100% sensitive. Hydrolysing the protein and estimating amino acids alone will give the exact quantification. The method developed by Lowry et al. is sensitive enough to give a moderately constant value and hence largely followed [10].

Estimation of total free amino acids content:

The amino acids are colourless ionic compounds that form the basic building blocks of proteins. Apart from being bound as proteins, amino acids also exist in the free form in many tissues and are known as free amino acids. They are mostly water soluble in nature. Very often in plants during disease condition the free amino acid composition exhibits a change and hence, the measurement of the total free amino acids gives the physiological and health status of the plants [5].

Extraction of total lipid content:

Lipids are soluble in some organic solvents. This property of specific solubility in nonpolar solvents is utilized for extracting lipids from tissues. In biological materials, the lipids are generally bound to proteins and they are, therefore, extracted either with a mixture of ethanol and diethyl ether or a mixture of chloroform and methanol. Inclusion of methanol or ethanol in the extraction medium helps in breaking the bonds between the lipids and proteins [9,11].

Estimation of free fatty acids content:

The free fatty acids in an oil is estimated by titrating it against KOH in the presence of phenolphthalein indicator. The acid number is defined as the mg KOH required to neutralize the free fatty acids present in 1g of sample. However, the free fatty acid content is expressed as oleic acid equivalents [5].

Micro-nutrient Analysis

Mineral estimation:

Preparation of sample for mineral analysis: Biological samples must be appropriately processed before they can be subjected to mineral analysis. There are three methods generally employed for processing the sample prior to mineral analysis as follows:

1. Ashing
2. Wet Digestion
3. Direct solution

The ashing method was employed for the analysis. According to this method the entire organic matter (if the tissue is destroyed) and the non-combustible material is recovered as ash. The minerals are then obtained / collected from the ash with an acid (usually dilute HCL), filterted and diluted to a known volume with deionized water and estimated quantitatively. In this method the organic compounds in the sample are decomposed by incineration at high temperatures (500°C-600°C) for 4-12 hr using muffle furnace.

Estimation of calcium by EDTA method: For the estimation of calcium ions the presence of Mg^{2+} ions is required. The dye Erichrome black-T preferably combines these Mg^{2+} ions to form a pink coloured Mg – dye complex. During the titration, EDTA first combines with free Ca^{2+} ions in the solution, and finally EDTA extracts at endpoint Mg^{2+} from complex. This results in the formation of free uncomplexed dye which in alkaline medium gives blue colour at the endpoint [12].

Estimation of phosphorus by Fiske-subbarow(ANSA) method: Ammonium acid molybdate reacts with inorganic phosphorous to form phosphomolybdicacid. The Mo^{6+} of phospho molybdic acid is then reduced to Mo^{4+} by means of reducing agent like 1- amino – naphthol – 4- sulfonic acid (ANSA) , to give deep blue coloured compound which is estimated colorimetrically. The reducing agent ANSA has only a negligible effect on the Mo^{6+} ions present in the unreacted acid molybdate reagent [9,13].

Estimation of iron by Wong's (KCNS) method: The ferrous ions present in the sample are oxidized to ferric ions by $K_2S_2O_8$ solution. The ferric ions give a red coloured ' ferro-sulphocyanide complex' with KCNS . The intensity of the coloured complex so formed is then estimated colorimetrically at 425 nm [14,15].

Estimation of magnesium by titan yellow method: Titan yellow reacts with magnesium in alkaline medium and gives an orange red colored complex. The intensity of the coloured complex so formed is then estimated colorimetrically at 540 nm. The intensity of the colour produced is proportional to concentration of magnesium. The procedure was developed by Neil and Neely [16,17].

Estimation of vitamins:

Estimation of thiamine by thiochrome method: Alkaline potassium ferricyanide oxidizes thiamine to thiochrome which is a fluorescent compound. The thiochrome is extracted in isobutyl alcohol and measured in a Fluorimeter [5].

Estimation of riboflavin: Riboflavin is used in veterinary and medical practices for supplementation of animal feeds and as natural coloring agent in food products. It is estimated in urine and with an average diet, the daily losses amount to 12% of the intake. A fall in the level of Riboflavin excretion occurs before deficiency symptoms of Vitamin B_2 are noticed. Under protein deficiency the urinary output of Vitamin B_2 increases. Riboflavin fluoresces at wavelength 440 nm to 500 nm. The intensity of fluorescence is proportional to the concentration of Riboflavin in the solution. The Riboflavin is measured in terms of the difference in fluorescence before and after chemical reduction [5].

Estimation of niacin by cyanogen bromide method: Niacin reacts with cyanogen bromide to give a pyridinium compound which undergoes rearrangement yielding derivatives. These derivatives couple with aromatic amines to give yellow colored pigment. Under proper conditions the intensity of the yellow color produced is proportional to the amount of Niacin present [5].

Estimation of ascorbic acid (Vitamin C) by DNPH method: Ascorbic acid is first dehydrated by bromination. The dehydroascorbic acid is then reacted with 2, 4-Dinitriphenyl hydrazine to form osazones and dissolve in sulphuric acid to give an orange red color solution which is measured colorimetrically at 540 nm [5].

Estimation of retinol (Vitamin A) by Carr-Price method: The retinol and carotenes are extracted into light petroleum by soxhlet method. It is than evaporated to dryness to obtain a residue which is reconstituted in n-Heptane. On addition of chloroform and Carr-price reagent, different intensities of blue color are obtained which is read at 465 nm [12,18,19].

Estimation of tocopherol (Vitamin E): Vitamin E activity is shown by four naturally occurring tocopherols of which œ -Tocopherol is the most potent tocopherol give Emmeric-Engel reaction which is based on on a reduction by tocopherols of ferric to ferrous ions which then form a red complex with œ, œ'-Dipyridyl. Tocopherols and carotenes are first extracted into xylene and the extinction read at 460 nm to measure the carotenes. A correction is made for these after adding ferric chloride and reading at 520 nm [12,20].

RESULTS AND DISCUSSION

The results of the biochemical analysis of various nutritional parameters viz., proximate principles and micronutrients are represented in Tables 1 and 2, respectively.

Table 1: Proximate principles of leaf extracts of *Moringa oleifera* Lam.

Sr. No.	Biochemical parameters	Values (g/100 g dry wt.)
Proximate Principles		
1.	Total Carbohydrate content	9.056 ± 0.14
2.	Total Reducing sugar content	3.495 ± 0.11
3.	Total Glucose content	1.924 ± 0.12
4.	Total Cellulose content	11.19 ± 0.21
5.	Total Crude fibre content	9.52 ± 0.31
6.	Total protein content	2.5 ± 0.19
7.	Total Free amino acids content	0.1198 ± 0.53
8.	Total Lipid/Fat content	1.82 ± 0.33
9.	Total Free fatty acid content	0.19 ± 0.06

*All values are expressed as mean ± SD for three determinations

Table 2: Micronutrients of leaf extracts of *Moringa oleifera* Lam.

Sr. No.	Biochemical parameters	Values (mg/100 g dry wt.)
Micronutrients		
1.	Total Calcium content	480.96 ± 0.27
2.	Total Phosphorus content	14 ± 0.12
3.	Total Iron content	34.4 ± 30.10
4.	Total Magnesium content	1416.0 ± 28.64
5.	Total Thiamin content	0.0187 ± 0.0028
6.	Total Riboflavin content	0.092 ± 0.022
7.	Total Niacin content	0.290 ± 0.019
8.	Total Ascorbic acid content	516.66 ± 76.98
9.	Total Retinol content	110.41 ±7.54
10.	Total Tocopherol content	39.545 ± 4.87

*All values are expressed as mean ± SD for three determinations

Since many of the herbal products are used orally, knowledge of proximate and nutrient analysis of these products and raw materials used there in plays a crucial role in assessing their nutritional significance and health effects [3,21,22].

The biochemical analysis of the leaf extracts of *Moringa oleifera* Lam. (MOL) showed considerably high levels of most of the estimated nutritional elements. The micronutrients analysis of the leaf powders of the *Moringa oleifera* Lam. (MOL) showed significant variation among different micronutrients. Magnesium content was found to be the highestfollowed by calcium, as compared to the rest of the tested minerals. However, phosphorus and iron contents were found to be comparatively less. Phosphorus content was found to be the least among all the tested minerals. Most of the vitamins were also found to be in good quantity. Aslam et al. in their study, have determined the mineral composition of *Moringa oleifera* Lam. leaves and pods from different regions of Punjab, Pakistan [23]. The result of their study has revealed that the pods and leaves of *Moringa oleifera* Lam., indigenous to different agro-climatic regions of Punjab, contained a considerably high amount of most of the minerals estimated by them, which included - Ca, Mg, K, Mn, P, Zn, Na, Cu and Fe and hence it was concluded by Aslam et al. that the pods and leaves of *Moringa oleifera* Lam. might be used as a viable supplement of dietary minerals [23]. The results of our study are found to be in accordance with the report of Aslam et al. and indicated that the *Moringa oleifera* Lam. leaf extract was found to be a good source of all the four minerals (Ca, P, Fe and Mg) as they were found to be in higher amounts in MOL [23].

CONCLUSION

From the results of the study, it can be concluded that the leaf powders of *Moringa oleifera* Lam. was found to be having all the essential nutrients, minerals and vitamins in good amounts and possess goodnutritive value. These plant leaf powders can thus be looked forward as the probable sources of food supplementation in future, after further investigation of the anti-nutritive factors present in them and their enzymatic and molecular effect on human health.

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