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ESTIMATION OF IMMUNOMODULATORY AND ANTI-DIABETIC MICRONUTRIENTS FROM SIX DIFFERENT LEAFY VEGETABLES OF BENGAL

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ABSTRACT: Micronutrients are essential nutritional elements that assist the body in synthesizing enzymes, coenzymes, prosthetic groups, hormones, and other compounds important for living beings' progress and expansion. Trace elements are one of the important part of micronutrients. Trace elements have an important impact on human health and have a wide range of metabolic properties and performance, although these elements constitute a small part of the biological tissues. Immune modulation by humoral and cellular mechanisms, nerve conduction, muscular contractions, membrane potential regulation and mitochondrial functioning and enzyme responses are just a few of the activities that trace elements perform in tissue, cellular and subcellular processes. In type 2 diabetes, selenium, chromium, zinc, calcium, magnesium, sodium, manganese, cobalt, iodine and iron levels appear to be low, iron and vanadium deficient, whereas potassium and copper levels remain unaffected. Numerous different micronutrients, such as zinc, iron, copper and selenium, are now required by the complicated, interconnected immune system that plays significant, often complementary roles at each and every stage of the immune response. This research is focused on micronutrients, such as zinc, selenium and chromium, playing an important role as anti-diabetic and immunomodulatory agents and their availability in the edible parts of green leafy vegetables of West Bengal.

INTRODUCTION: Trace elements are essential micronutrients for the correct functioning of human body as they significantly impact metabolic and physiological processes. Micronutrients are composed of trace minerals, macro-minerals, vitamins, and organic acid. Iron, copper, zinc, manganese, iodine, chromium, and selenium are trace elements or minerals, while calcium, phosphorus, potassium, sodium, and magnesium are significant macro minerals for humans.

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These micronutrients undertake various biochemical activities in the human body; their absence can lead to malfunctioning, organ failure, and the emergence of non-communicable diseases like diabetes, lowering the immune system, cardiovascular disease and cancer. Aside from providing trace elements and vital minerals in the form of tablets or capsules, the approach to avoiding such deficits includes a continuous investigation of unique micronutrient-rich components of the human diet.

Leafy vegetables may be a potential alternative source of trace elements [1-3]. Natural compounds, especially those of plant sources, are the primary target for identifying viable lead compounds and will play a key role in novel drug development initiatives [4-6].

Plant-based preparations are the major fundamental player in the world of medications because of their ease of availability, low cost, and low side effects [4]. Many plants contain various bioactive compounds with significant pharmacological benefits but no/minimal harmful side effects [4, 7]. Several presently available drugs originate actively or passively from plants, which have been good sources of traditional medicine and are well known for their folklore use since long ago [4, 8, 9]. Bengal has long been known for medicinally rich plants, and many of them are used traditionally for different diseases and disorders. Among them, a large part of them are green leafy vegetables, which are part of our daily diet. Leafy vegetables exhibit anti-diabetic and immunomodulatory activity because of their secondary metabolites and/or micronutrients [2, 10].

Apiaceae, Convolvulaceae, Acanthaceae, Brassicaceae, Amaranthaceae, and Rutaceae are the major leafy green vegetable families in West Bengal that contain the significant phytoconstituents with anti-diabetic activity and immunity-boosting properties. Diabetes is a long-term condition in which the pancreas fails to deliver sufficient insulin or the body's insulin is inefficiently utilized. Diabetes prevalence among persons aged 20 to 70 is expected to rise to 8.3% in 2013 and 10.1% in 2035 worldwide. Hyperglycaemia claimed the lives of an estimated 5.1 million individuals worldwide in 2013 [11]. Diabetes is expected to be the sixth cause of death by 2030. The Mideast region, Sub-Saharan Africa, and India are expected to see the biggest relative growth [4, 14, 15].

Diabetes advancement, particularly poor glycaemic management, leads to a slew of possibly deadly consequences. Diabetic patients account for about half of all people with chronic renal disease. Similarly, 9.8% of diabetics have had a heart attack, 9.1% have cardiovascular disease (CAD), 7.9% have congestive heart failure and 6.6% have had a stroke, while more than a significant proportion of them (27.8%) have diabetic nephropathy, nearly a quarter (22.9%) have foot issues and last of all, 18.9% have eye injuries [4,12,13]. Our bodies are overwhelmed with pathogens whose primary mission is to thrive and reproduce in a warm, wet, nutrient-rich environment from the time we are born. Many organisms survive and grow by employing highly specific processes that allow them to invade the body, proliferate in nutritionally appropriate environments and then escape to infect a new host. These mechanisms result in illness and disability in the body. Physical and biochemical barriers, specialized immune cells, and antibodies targeting pathogenic microbes make up the complex immune protective mechanism used to attack harmful pathogens [16, 17, 18]. Increase the immunity is required for our general health and also for fighting against the covid-19 virus. In the recent era, COVID-19 quickly became a global pandemic, posing lethal hazards to overburdening healthcare resources [19]. The immune system must be in good working order to prevent the disease from progressing to this stage and to reduce the harm caused by the coronavirus (SARS-CoV-2).

Nutritional health is essential for effective immune protection and, as a result, a satisfactory response to SARS-CoV-2 [20]. Not only covid-19, but a Lack of good immunity may also facilitate to other diseases and disorders. Micronutrients help to promote the immune system's multiple aspects, including physical barriers, cellular responses, and antibody synthesis. Insufficient or poor micronutrient intake has a negative impact on the body's capabilities, reducing its overall ability to fight diseases [21]. Nowadays, diabetes is a prevalent disease all over the world, and we are all facing a pandemic due to SARS-coV-2. Hence maintaining proper blood sugar levels and boosting our immunity is essential and has become one of the thrusts of present pharmaceutical research.

Trace elements contents of green leafy vegetables are widely available in our surroundings and are commonly intake by the people of Bengal. Hence these can be the potential solution to the crisis of immunity and diabetes. However, quantitative profiling of trace elements among leafy vegetables of Bengal has not yet been established completely [22, 23].

Therefore the objective of the present study was the qualitative and quantitative estimation of the trace elements which have anti-diabetic and immunomodulatory properties from sleeted commonly available six leafy vegetables of West Bengal.

MATERIALS AND METHODS:

Plant Materials: Six leafy vegetables were selected:

- *Coriandrum sativum L* (coriander leaves)., family: Apiaceae
- *Hygrophila auriculata* (Schumach.) Heine (kulekhara), family: Acanthaceae
- *IpomoeaaquaticaForsk (*Kalmishak*),* family: Convolvulaceae
- *Spinacea oleracea L* (Palongshak), family: Amaranthaceae
- *Brassica juncea* (L.) (Soresshak), family: Brassicaceae
- *Murraya koenigii* (L.) Spreng (curry leaves). family: Rutaceae

- **Chemicals:** Concentrated Nitric acid (HNO_3), Perchloric acid ($HClO_4$), and Concentrated Hydrochloric acid (HCL).
- **Instruments:** AAS (Atomic Absorption Spectroscopy) (model no: Perking Elmer, pin AAcle 500), Hot plate, Muffle furnace.
- **Sample Collection:** Leafy vegetables are collected from the local market of Habra, West Bengal.
- **Sample Preparation:** Freshly acquired green vegetables were shade dried before being pulverized and placed in a pre-clean, labelled container for subsequent analysis.
- **Determination of Trace Elements Contents:** Decomposition of the sample is required for several analytical procedures, including AAS for trace element identification in plant materials. As a result, the mineralization technique is essential for getting desirable analyte values [24]. Before identifying the entire inorganic component in plant substances (raw powdered sample), it is normally essential to remove the organic matter. The methods for destroying organic materials can be divided into "Wet Oxidation" and "Dry Ashing."Method [25].

A. Wet Oxidation Method: 0.5 gm. raw plant powdered samples were taken and digested on a hot plate at low temperature (55-70^{o}c) for about 30 minutes with 10 ml of concentrated nitric acid (65%). After cooling the sample mixture to room temperature, 5 ml perchloric acid (70 to 72%) was added, and the contents were digested again until a clear solution was obtained. Cooled and diluted the sample with deionized water, filtered (if necessary), and transferred to a 100ml volumetric flask. Volume was made up of deionized water. AAS was used to determine the mineral content. The data were given in mg/L of the dry weight of the powdered sample [25].

B. Dry Ashing Method: For ash preparation, 5 gm of samples were held in a crucible for 1 hour at 500°C in a muffle furnace. The ash was then digested with an HCl and HNO_3 solution (1:3). The digested samples were dissolved in water and then filtered the sample and filled to a volume of 50 ml with double distilled water. AAS was used to determine the mineral composition. The values obtained in mg/L of dry weight of the powdered sample [26].

C. Preparation of Standard: For the blank 2% HNO_3 was prepared, and the calibration standard of Zn, Cr, and Se was prepared by the using Zn, Cr, and Se stock solution. Cr, Se, Zn can be estimated by AAS directly following the equipment's calibration method and estimation procedure.

RESULTS AND DISCUSSION: In this work, we looked for a pre-selection of unique, abundant dietary sources of important trace elements from various leafy vegetables in West Bengal that reduce hyperglycaemic levels and boost the immunity and, therefore, can be regarded as supplemental nutrient reservoirs. The existence on the market and consumers' popularity were deciding factors in the selection of the six leafy vegetables included in the study. Using the dry ashing and wet oxidation methods through AAS, six leafy vegetables belonging to the family Apiaceae, Acanthaceae, Convolvulaceae, Amaranthaceae, Brassicaceae, Rutaceae were evaluated concerning the Se, Cr, and Zn, focusing on their anti-diabetic and immunomodulatory properties.

Micronutrient Composition: Table 1 shows the micronutrient concentrations analyzed by the dry

ash method and the wet oxidation method. In this research, when a sample is prepared by the dry ashing method, leafy vegetables' micronutrient content is higher than the sample prepared by the wet oxidation method.

TABLE 1: CONCENTRATIONS OF MICRONUTRIENTS (mg/l) IN AERIAL PART OF SELECTED LEAFY VEGETABLES OF DIFFERENT BOTANICAL FAMILIES ANALYSED BY DRY ASHING METHOD AND WET OXIDATION METHOD (n = 3, MEAN ± SEM).

Sample name	Se (mg/L)		Cr (mg/L)		Zn (mg/L)	
	Dry Ashing method	Wet oxidation method	Dry Ashing method	Wet oxidation method	Dry Ashing method	Wet oxidation method
M. koenigii	3.069±0.017	0.850±0.017	0.664±0.015	1.916±0.017	12.05±0.036	0.915±0.002
B. juncea	3.413±0.056	1.009±0.013	1.685±0.008	0.921±0.032	12.71±0.012	2.451±0.011
I. aquatica	2.860±0.044	0.788±0.018	2.233±0.020	0.190±0.080	9.661±0.021	0.626±0.001
H. auriculata	2.796±0.066	0.802±0.035	1.611±0.010	0.232±0.046	12.35±0.001	1.133±0.002
S. oleracea L	4.159±0.159	4.142±0.010	1.226±0.016	0.064±0.012	11.43±0.047	1.720±0.010
C. sativum L	2.269±0.030	3.798±0.044	2.412±0.004	0.075±0.009	6.984±0.014	1.042±0.001

The trace elements are used to maintain the health of humans and other animals [11, 27]. Vitamins and macro-elements interact with trace elements to increase their impact on the body [11]. Insufficient trace element consumption can lower immunity and increase malnutrition, rendering patients more susceptible to illness. An investigation has indicated that particular nutrients preferentially regulate the immune response in humans. That insufficiency and overabundance can disrupt the coordinated host response to infections, and that shortage can change the pathogen city of otherwise harmless viruses [21].

Each step of the immune system depends on specific micronutrients, which play combinatorial roles due to their complementing different mechanisms of action. As a result, for the immune system to function correctly, micronutrients must be consumed in appropriate quantities [16]. The impact of micronutrient deficiencies and the potential value of supplementation in the prevention and management of type 2 diabetes is obvious micro/macronutrients are important in glucose metabolism [11]. The literature revealed that zinc and selenium help manage diabetes and help with immune function, but that Cr only helps manage diabetes. In this research, the concentration of Cr, Se, and Zn of selected six leafy vegetables analyzed by the dry ashing method and the wet oxidation method is shown in **Table 1**. Zinc is an essential element in the metabolism of glucose. It aids in the use of glucose by muscle and fat cells. It is a necessary cofactor for intracellular enzymes involved in protein, lipid and glucose homeostasis. Zinc may play an important role in controlling the insulin receptor-initiated signal transduction process and insulin receptor production [11]. For immunity, zinc is required for rapidly reproducing cells, particularly those in the immune system, and it regulates both innate and acquired immunological processes. It is a cofactor for thymulin, which helps regulate cytokine release and initiates proliferation, and it is implicated in the cytosolic defense against oxidative stress (superoxide dismutase interaction).

Unbound zinc ions have a direct antiviral effect on rhinovirus replication, and enough zinc consumption stimulates a Th1 response and maintains skin and mucosal membrane structure [21]. **Table 1** explored that zinc concentration analyzed by dry ashing method from six leafy vegetables are variable. Concentration was found to be highest 12.7 mg/L belonging family Brassicaceae *(B. juncea)*, and the lowest concentration was found 6.984 mg/L belonging family Apiaceae *(C. sativum L)*. Zinc concentration analyzed by wet oxidation method, the highest concentration is 2.45 mg/L belonging to the family Brassicaceae *(B. juncea)*, and the lowest concentration is 0.626mg/L belonging to the family Convolvulaceae *(I. aquatica)* shown in **Table 1**. In both methods *B. juncea* belonging family Brassicaceae was evidenced to contain maximum zinc content. Selenium, an essential trace element, has a role in oxidative stress defense *via* selenium-dependent glutathione peroxidases and some other selenoproteins [11]. Selenium is necessary for a healthy immune response and impacts both the innate and acquired immune systems. Through glutathione peroxidases, which eliminate excess

potentially damaging radicals created under oxidative stress, it plays an important role in redox control and antioxidant activity. As a result, selenium is vital for maintaining redox balance and protecting the host from oxidative stress caused by macrophages' microbicidal activities and inflammatory responses [21].

Table 1 disclosed that selenium concentration analyzed by dry ashing method from six leafy vegetables are variable, found to be highest at 4.159 mg/L in the belonging family Amaranthaceae *(S. oleracea L)* and lowest 2.269 mg/L in the belonging family Apiaceae *(C. sativum L).* Selenium concentration analyzed by wet oxidation method from six leafy vegetables was found to be highest at 4.142 mg/L in *S.oleracea L* of the Amaranthaceae family and lowest at 0.788 mg/L in *I. aquatica* of family Convolvulaceae **Table 1**.

Selenium concentration was found to be high in both methods in S. oleracea L [Amaranthaceae]. Chromium is an important element of the glucose tolerance factor (GTF), which plays a significant role in glucose homeostasis and is essential for optimal carbohydrate metabolism and as an essential cofactor for insulin action [11].

Table 1 showed chromium concentration was maximum (2.412 mg/L) in *C. sativum L,* [Apiaceae] by dry ashing method, whereas minimum concentration was (0.664 mg/L) found in *M. koenigii* [Rutaceae]. By wet oxidation method, maximum chromium concentration was 1.916mg/L in the Rutaceae family [*M. koenigii*] and minimum concentration of chromium was 0.064mg/L in Amaranthaceae family [*S. oleracea L*] **Table 1**.

Micronutrient deficiencies are well-known to have a negative impact on the immune system and make people more susceptible to illnesses. Minor deficits are linked to a higher risk of infections, but the effect may be less pronounced than that seen with overt deficiencies. Several micronutrients are in short supply in the world's diets, particularly in affluent countries, increasing the risk of sickness. As a result, there is an imbalance between food intake and the levels required for healthy immune function, prompting micronutrient supplements to promote the immune system and minimize the risk of infection [16].

The incineration of plant material at relatively low temperatures (500°C) is commonly followed by disintegration in hot acid (HCl, HNO_3, or a mixture of both) in the dry ashing method. The "wet oxidation" method is the process of destroying leaves in acid ($HClO_4$ and HNO_3). The dry ashing method is more widely employed since it is easier, safer, less expensive, and more reliable than the wet oxidation method, which uses potentially carcinogenic or hazardous compounds [28]. However, it was observed in this study that when the sample was prepared by the dry ashing method, the Cr, Se, and Zn concentration of leafy vegetables of Bengal was comparatively higher than when the sample was prepared by the wet oxidation method. As per the requirement and availability, suitable leafy vegetables can be suggested based on the present study of trace elements for managing diabetes and boosting immunity.

CONCLUSION: The active elements of herbal and modern medicine can be found in leafy vegetables. Bengal has one of the richest herbal medical cultures in the world that is of tremendous contemporary relevance ensuring health security for millions of people. For a long back, Bengal people have taken several leafy vegetables as part of their daily diet and used them for the prevention of a wide range of disorders starting from common cold to cancer and snake poisoning therapy to cure for hereditary illnesses like neuromuscular disorders, because of these leafy vegetables are inexpensive and readily available. Although leafy vegetables are regularly ingested, trace element profiling has not yet been completed. The present study reveals a comparative outcome of three important trace elements *viz,* Se, Cr, and Zn concerning diabetes and immunity-related disorders from six commonly available and consumed leafy vegetables of West Bengal. However, future study on the estimation of the elements from remaining unexplored plants of Bengal will be helpful to complete the trace element profiling and be beneficial for the nutritional suggestion for the society.

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