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Evaluation of Protein and Carbohydrate Content of Some Anti-Diabetic Medical Plants

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Abstract: ***Diabetes mellitus may be defined as a state where the body of a person is deficient in the production of insulin. It may lead to a high amount of glucose in the bloodstream. The uses of medication for diabetes have many side effects. This paper involves a biochemical analysis of protein and carbohydrate content of Ocimum sanctum, Aegle marmelos, and Azadirachta indica leaves by use of extraction method. The plant samples for the same were collected from Vadodara and Anand district located in Gujarat. The results showed that the Azadirachta indica leaf sample collected from Anand showed maximum protein content and the Aegle marmelos leaf sample obtained from Vadodara had maximum carbohydrate content when compared with all plant samples collected for analysis. It may indicate that these plants have great anti-diabetic potential and can be used for the development of a potent drug from natural sources in the future.***

Keywords: Ocimum sanctum, Aegle marmelos, Azadirachta indica, Anti-diabetic

I. INTRODUCTION

Medicinal plants are used for their therapeutic as well as health-promoting benefits. They are used as a natural cure for the treatment of several diseases that can affect the bodily function of humans as well as animals. The main aim of making use of plants is for medicinal benefits. They have long been used for treatment due to the presence of a substance that can be used for the formation and synthesis of various drugs. These plants contain compounds that give relief from indicative problems. Some of the most valued medicinal plants are used for the prevention as well as to manage diabetes. The presence of various elements in plants like alkaloids, minerals, tanin, terpenoid, phenolics, anthraquinones, flavonoids, etc. is a few compounds that make the therapeutic in nature. The restorative action on the injured tissue of the liver, pancreas, and other diabetes-related organs may be due to their corrective action.

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The healthy organs and their cellular tissue like hepatic tissues and beta-pancreatic cells are responsible for the protective action. It may also be due to inhibitory action on inducers on diabetes [1]. Diabetes mellitus can be described as the lack of insulin production in the human body. It may be a chronic disorder of supermolecule metabolism, saccharide, and fat distinguished by high glucose levels in the bloodstream of the human body. In diabetic conditions, an individual's body cannot use the insulin it produces as it is lowered and the body may not be able to produce enough endocrine. Over time this may lead to a medical condition called hyperglycemia. It is a kind of metabolic disorder. The most commonly observed symptoms in diabetes may include vision loss, more urge to urinate, tiredness, feeling thirsty, increased hunger, etc. [2]. Diabetes can be mainly categorized as type 1, type 2 as well as Gestational diabetes. In type 1 diabetes which is also called juvenile diabetes, there is insufficient production of insulin in the body. In this Beta cells also called the insulin-producing cells present in the pancreas are damaged by the immune system. As a result of which the flow of glucose into the cells is stopped as the body does not produce enough insulin. This stints the cells. It is also called insulin-dependent diabetes. It is believed to be caused by an autoimmune reaction where the hypoglycaemic agent may be stopped producing in the body. The symptoms of type 1 diabetes may develop very quickly and may include fast heart rate, headaches, sudden weight loss, increased infections, etc. To survive a person may take a hypoglycaemic agent in this type of diabetes. Type 2 diabetes occurs when the cells of the body stop responding to the insulin produced in it the way it generally does. This leads to resistance towards insulin hormone. It is a circumstance that the body can't process the sugar it produces into energy. It is also the most common type of diabetes and is also known as Adult-onset diabetes. Factors that increase the risk of this include family history, inactivity, weight issues, etc. the common symptoms of type 2 diabetes may include slow healing sores, numbness of hands or the feet, darkened skin areas like neck and armpits, etc. It is also called non-insulin-dependent diabetes. Gestational diabetes is caused due to changes in hormones during the onset of pregnancy. This may be caused due to high level of blood glucose levels in pregnant women. People who have such conditions might develop type 2 diabetes in the future and no symptoms may be observed in this diabetes [3], [4], [5]. *Ocimum sanctum* also called the holy basil is commonly called Tulsi in Hindi. It belongs to the family Lamiaceae is grown for its aromatic leaves. It is an asterid dicot belonging to an angiosperm. It is used in folk as well as ayurvedic medication systems. It may be used as herd tea to cure various sicknesses at times.

The plant lowers blood pressure has hypoglycaemic properties and is a very helpful herb for human beings. It also possesses anti-diabetic, antioxidant, anti-bacterial, and anti-fungal properties [6].

Azadirachta indica also called margosa and Neem in Hindi is an angiosperm that belongs to the Meliaceae family is a medicinal plant. It has many pharmacological uses. Its oil is also used as a repellent. For a long time, it has been used for the treatment of diabetes and is also used in cosmetics. It is a highly utilized medicinal plant and possesses anti-diabetic properties [7].

Aegle marmelos called bael in Hindi is a member of Rutaceae and is a dicotyledon. It is an angiosperm. It is an important plant of the Siddha system of medicine. It is known for its anti-diabetic properties and also contains hypoglycaemic benefits. The leaves of this plant are used for the treatment of various medical problems and it acts as an antioxidant plant [8].

II. MATERIAL AND METHODS

A. Collection of Plant material and Soil

The fresh and healthy leaves of *Ocimum sanctum*, *Azadirachta indica*, and *Aeagle marmelos* were collected from two different places named Anand (Latitude 22.5645° N, Longitude72.9289° E) and Vadodara (Latitude 22.2726°N, Longitude73.1878° E) situated in the state of Gujarat. The collected sample was washed well to remove sand and dust and leaves were separated. The separated leaves were shade dried and made into a fine powder using a mixer grinder and powder form was used for further analysis.

B. Preparation of plant extracts

From both sites the powdered form of *Ocimum sanctum* was mixed with methanol, *Azadirachta indica* was mixed with glacial acetic acid and *Aeagle marmelos* was mixed with distilled water in beakers. Six tripod stands were placed alongside each other and a funnel was placed at top of each stand. The filter paper was placed at the mouth of all funnels. The mixed leaf samples from both the town were then added to the funnel and allowed to pass through the filter paper at the mouth of the funnel. The extracted liquid was then collected into beakers placed at bottom of each tripod stand and the mouth of the beakers was covered using aluminum foil. The collected extract was then used for performing the experiments [9].

C. Determination of Quantitative Biochemical analysis

Quantitative analysis is the method that is used to find out the amount of a particular component in the given sample or substance. It is detailed profiling used to identify the composition of contents in a particular sample. It can be performed via various analytical methods depending upon its application in a different field. It helps in the determination of total yield as well as contamination or impurities present in a sample. It is also done to check whether a component is present under the limits specified. Biochemical analysis of plants is done to find out the various substances present in the plant and study their composition [10].

D. Determination of Total protein content

Requirements: BSA (*Bovine Serum Albumin*) stock solution, Analytical Reagent, Folin's Reagent, Burette, spectrophotometer, beaker, test tube stands, test tubes, distilled water, pipette, measuring cylinder & a marker.

Proteins are one of the most important components of the plant. They utilize them for various roles like biosynthesis, immunity, etc. In the protein by Lowry method for determination of protein four test tubes were taken and labeled with markers as 0.2ml, 0.4ml, 0.6ml, and 0.8 ml. To this series of aliquots, standard BSA (*Bovine Serum Albumin*) stock solution was added carefully with help of a pipette as per label. Another set of three test tubes was taken and labeled as Unknown1, U2, and U3. To these test tubes 0.2ml, 0.4ml, and 0.6 ml of the extracted samples of *Ocimum sanctum*, *Azadirachta indica*, and *Aeagle marmelos* from both the sites were added. After this, the final volume was made up to 1ml using distill water by using a pipette. Another test-tube labeled as blank was also prepared and 1ml of distilled water was added to the same with a pipette. All the test tubes were then carefully placed in the test tube stand. With help of a pipette 2ml of alkaline copper, sulfate reagent was added to each test tube. The test tubes were then allowed to rest for 10 minutes at room temperature. After that 0.5 ml of Folin's reagent was added to each test tube with a pipette. The test tubes were then placed

into a dark room at room temperature for about 30 minutes. They were then taken out and the Optical Density (OD) of all the test tubes was taken at 660 nm with help of a spectrophotometer [11].

E. Determination of Total Carbohydrate Content

Requirements: Spectrophotometer (UV), Stock Solution (Standard *Glucose*), Anthrone Reagent, beaker, test tube stands, test tubes, distilled water, pipette, Water Bath/ Mantle heater & a marker. Carbohydrates are important for giving strength to plants and are present in form of cellulose. They are mainly utilized by plants for food production. They also use it for the process of respiration. Five test tubes were taken and labeled with markers as 0.2ml, 0.4ml, 0.6ml, 0.8 ml, and 1.0ml. To this series of aliquots, Stock Solution (*Standard Glucose*) was added carefully with help of a pipette as per label. After this, the final volume of this series was made up to 1ml with distilled water using a pipette. Another set of two test tubes was taken and labeled as Unknown1 and Unknown 2. To these test tubes 1ml of the extracted samples of *Ocimum sanctum*, *Azadirachta indica*, and *Aeagle marmelos* from both the sites were added. Another test-tube labeled as blank was also prepared and 1ml of distilled water was added to the same with a pipette. All the test tubes were then carefully placed in the test tube stand. With help of a pipette 3ml of Anthrone Reagent was added to each test tube. All test tubes were then placed in the water bath for about 7 minutes. Then 4ml of distilled water was added to each test tube. After that Optical Density (OD) of all the test tubes was taken at 620 nm with help of a spectrophotometer (UV) [12].

III. RESULT

Ocimum sanctum, *Aegle marmelos*, and *Azadirachta indica* are exudates material obtained from the plant leaves. Phytochemical parameters were estimated based on the standard methods [9], [10], [11], [12].

A. Determination of Total Protein Content (TPC)

The results of total protein content determination are given in the table below. (Table-I) Results indicated different total protein content of *Ocimum sanctum*, *Aegle marmelos*, and *Azadirachta indica* leaves. The result of the determination of total protein content is given in a graphical presentation (Fig. 1).

B. Determination of Total Carbohydrate Content (TCC)

The results of total carbohydrate determination are given in (Table-II) Results indicated different total carbohydrate content of *Ocimum sanctum*, *Aegle marmelos*, and *Azadirachta indica* leaves. The result of the determination of total protein content is given in a graphical presentation (Fig. 2).

Table-I: Total Protein Content of Ocimum Sanctum, Aegle Marmelos, and Azadirachta Indica in Terms Of Bovine Serum Albumin (Bsa) Equivalents

Determination Total Protein Content			
City	**Ocimum sanctum**	**Azadirachta indica**	**Aegle marmelos**
Vadodara	79.05 ± 0.04 mg of BSA/g	90 ± 0.20 mg of BSA/g	101 ± 0.12 mg of BSA/g
Anand	101.2 ± 0.12 mg of BSA/g	110.1 ± 0.14 mg of BSA/g	98.25 ± 0.18 mg of BSA/g

All the Values are expressed as Mean ± SEM (Standard Error of Mean) (n=3); BSA= Bovine serum albumin

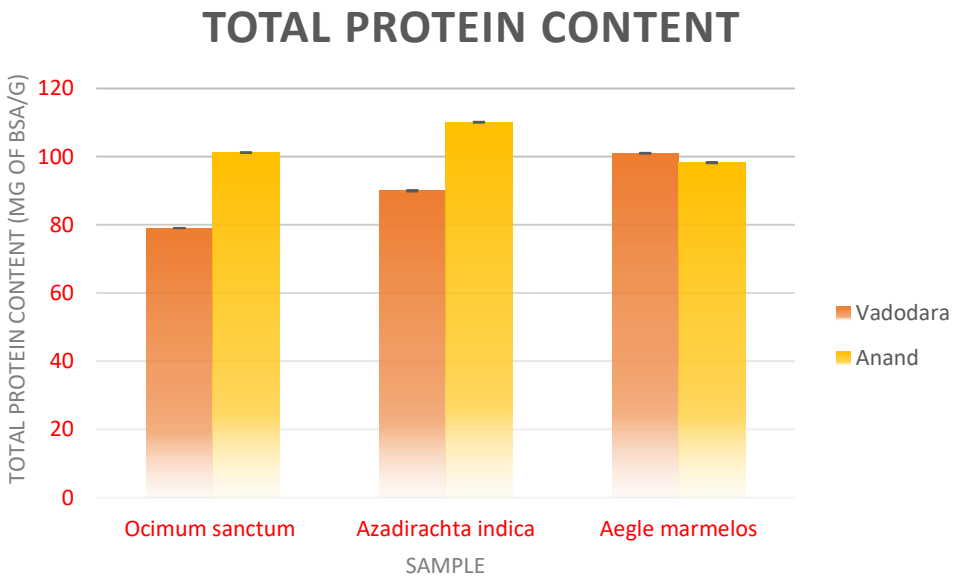

Fig. 1: Total Protein Contain

Table-II: Total Carbohydrate Content of Ocimum Sanctum, Aegle Marmelos, And Azadirachta Indica in Terms of Glucose Equivalents

Determination Total Carbohydrates Content			
City	**Ocimum sanctum**	**Azadirachta indica**	**Aegle marmelos**
Vadodara	80.5 ± 0.18 mg of GUL/g	108.05 ± 0.78 mg of GUL/g	150 ± 0.08 mg of GUL/g
Anand	92.3 ± 0.24 mg of GUL/g	69 ± 0.81 mg of GUL/g	75.1 ± 1.01 mg of GUL/g

All the Values are expressed as Mean ± SEM (Standard Error of Mean) (n=3); GLU=Glucose

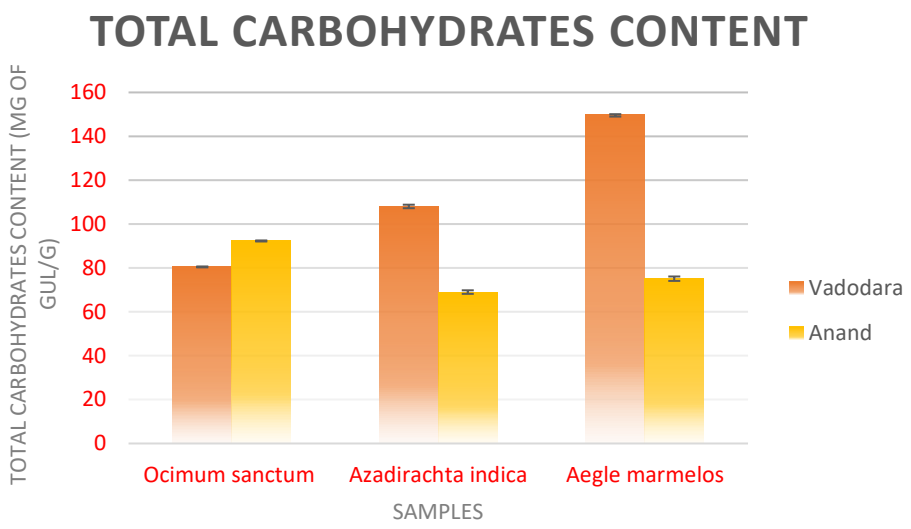

Fig. 2: Total Carbohydrates Content

IV. DISCUSSION

Bovine serum albumin (BSA) is the typical recommendation for total macromolecule quantitation by colorimetric analysis. Expedient BSA standards are designed for serial dilution to get correct standard curves and square measure exactly formulated at 2mg/ml. BSA is employed due to its steadiness to extend signal in analysis, its lack of impact in several organic chemistry reactions, and its low price since massive quantities of it may be promptly pure from bovine blood, a probe for the cattle trade [13].

The total protein content of thirty-three edible plant leaves including species *Azadirachta indica* and *Aegle marmelos* taken from August to September month from various regions of Kolkata and it was found that the concentration of protein in *Azadirachta indica* was found to be 70.72±1.39 mg BSAE/g of fresh weight and that of *Aegle marmelos* was found to be about 84.73±1.55 mg BSAE/g of fresh weight, whereas the readings obtained for the protein concentration of *Azadirachta indica* was found to be 90±0.20 mg BSA/g and 101±0.12 mg BSA/g for Vadodara and Anand respectively and that of *Aegle marmelos* was found to be 110.1±0.14 mg BSA/g as well as 98.25±0.18 mg BSA/g for Vadodara and Anand. On comparing with the former, the concentration of protein is higher in the plant leaf samples of Azadirachta indica and Aegle marmelos taken from Vadodara and Anand, which points out the fact that they are present in more quantity in these plants [14].

A comparative study of seed storage profiling of selected species of the genus vigna was conducted at Badnapur, India. The total protein content estimation for ten different species of the vigna family was also conducted. It was revealed that the protein content was found to be between 36.23 mg of BSA/g to 57.5 mg of BSA/g. The protein estimation for our plants revealed that the total protein content in leaves of plant *Osmium sanctum* was found to be about 79.05±0.04 mg of BSA/g and for 101.02±0.12 mg of BSA/g and that of *Azadirachta indica* was found to be 90±0.20 mg BSA/g and 110±0.14 mg BSA/g for Vadodara and Anand respectively. Similarly, the value of protein content of *Aegle marmelos* leaves was found to be 101.1±0.12mg BSA/g as well as 98.25±0.18 mg BSA/g for Vadodara and Anand. From this, it can be concluded that all the leaf samples from both Vadodara and Anand contain more protein in general. The value of protein content for *Azadirachta indica* leaves obtained from Anand was higher in comparison to the seed samples from Badnapur, India which may be attributed to the physiochemical contents present in that plant [15].

The protein content of *Osmium sanctum* leaves from the extracted sample obtained from Vadodara was found to be 79.05±0.04 mg of BSA/g and that from Anand was found to be 101.2±0.12 mg of BSA/g. Similarly, for *Azadirachta indica* leaf extract the values obtained were 90±0.20 mg of BSA/g and 110.1±0.14 mg of BSA/g for Vadodara and Anand and that of *Aegle marmelos* was found to be 101±0.12 mg of BSA/g for Vadodara and 98.25±0.18 mg of BSA/g for Anand. The protein concentration was found to be maximum for the leaf extracts of *Azadirachta indica* obtained from Anand about 110.1±0.14 mg of BSA/g indicating that protein is present in the highest amount in a sample obtained from Anand and the lowest concentration was found to be from plant leaf extract of *Osmium sanctum* 79.05±0.04 mg of BSA/g taken from Vadodara which may be due to dominance of other compounds present within the plant or the contents present in the soil. Glucose is a simple type of sugar commonly called a monosaccharide. In both plants and animals, sugar can act as a signaling molecule and as a nutrient as a result of which carbohydrate composition can vary based on various factors like age, nutrition status time of day, etc. Particularly in plants sugars play a very important role by acting as a trigger of many processes, biotic and abiotic responses, etc [16]. The use of glucose as a standard is helpful since it has high specificity for carbohydrates making it an important factor for analyzing plant extracts.

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By use of glucose certain amount of information may also be obtained about the type of carbohydrate that may be present in the extract by use of the anthrone method [12].

Insulin resistance is when the glucose is unable to enter the cells of the body. When the pancreas is unable to produce enough insulin its ability to act as a key to allow glucose into cells stops as a result of which glucose can't move out of the blood which is then detected as increased blood glucose levels in our body. There may be very little or absent endogenous beta-cell performance, insulin treatment is important to forestall diabetic ketoacidosis, and also its aims to be precise replacement of insulin within the fasting state and once meals. However, insulin is required to relocate glucose into cells or within it, while glucose is held on and later used for energy. If an individual has a pair of diabetes, their fat, liver, and muscle cells don't respond properly to insulin [17].

Some phytochemical analysis of leaf extract of *Azadirachta indica* from Minna, Bosso Campus, Niger State. It also included the analysis of carbohydrates in the leaf extract. The result obtained for the carbohydrate analysis of plant leaf extract showed that 78.12±0.35 mg of GUL/g was present in the sample. The reading obtained for the samples of leaf extract of plant taken from Vadodara and Anand was found to be 108.5±0.78 mg of GUL/g and 69.0±0.81 of GUL/g respectively. It indicates that the sample from Vadodara contains more carbohydrates and that Anand contained less carbohydrate in comparison to the former which can be attributed to the fact that other constituents in the leaves varied in both cases [18].

Estimation of some phytoconstituents and evaluation of antioxidant activity in *Aegle marmelos* leaves extract was done. The sample of leaf extract for the same was collected from Allahabad, Uttar Pradesh.it was found that the total carbohydrate concentration was found to be about 34.53±0.570 mg of GLU/g. In the case of the sample of leaf extracts of *Aegle marmelos* from both Vadodara was found to be about 150±0.08 mg of GLU/g and that of Anand was found to be 75.1±01.01 mg of GLU/g. Hence, the value of total carbohydrate content for samples of leaf extracts of both Vadodara and Anand is greater in comparison to the value obtained from the leaf samples collected from Allahabad, Uttar Pradesh. It indicates that the plants present in the latter locations are richer and carbohydrate is one of dominating nutrients in them [19].

The Phytochemical investigation and in vitro antioxidant evaluation of some *Ocimum sanctum* species was conducted. This experiment was conducted on various species of *Ocimum* including *Osmium sanctum*. The leaf samples for the same were collected from Baipriguda of Koraput district (India). The carbohydrate content estimation of the species was done and it was found that the *Osmium* sanctum leaf contained 57.52 mg of GLU/g of carbohydrate. The values of the sample of a leaf extract from Vadodara and Anand for carbohydrates is 80.5±0.18 mg of GLU/g and 92.3±0.24 mg of GLU/g. Hence it can be concluded that the value of carbohydrates obtained for leaf samples of *Osmium sanctum* extract is greater and that of Anand is even higher which may be attributed to the physiochemical constituents present in the plant [20].

The total carbohydrate content for extract sample of *Osmium sanctum* from Vadodara and Anand was found to be about 80.5±0.18 mg of GLU/g and 92.3±0.24 mg of GLU/g respectively whereas the carbohydrate concentration for the *Aegle marmelos* plant leaf extract was found to be 150±0.08 mg of GLU/g for Vadodara and that of Anand was found to be 75.1±01.01 mg of GLU/g. Similarly, the carbohydrate concentration of plant leaf extract of *Azadirachta indica* from Vadodara and Anand was found to be 108.5±0.78 mg of GUL/g and 69.0±0.81 of GUL/g respectively. From the following, it can be seen that the extracted leaf sample of *Azadirachta indica* collected from Vadodara had the highest carbohydrate content of all leaf extracts which can be due to its physiochemical composition and the lowest carbohydrate content was found to be in the extract of *Azadirachta indica* sample collected from Anand attributed due to the dominance of other nutrients or the contents present in the soil.

V. CONCLUSION

The biochemical analysis of *Ocimum sanctum*, *Aegle marmelos*, and *Azadirachta indica* was done by extraction to find its protein and carbohydrate content to evaluate its anti-diabetic potential. When compared with all plant samples analyzed, it was found that Protein content was highest for the *Azadirachta indica* sample with Glacial acetic acid obtained from Anand and lowest in the *Ocimum sanctum* sample with methanol obtained from Vadodara which might be due to dominating nature of other compounds in the plant. Similarly, on analyzing all the samples for Carbohydrate content it was found that sample of *Azadirachta indica* plant with glacial acetic acid obtained from Vadodara had a high value of carbohydrate content and lowest for the sample of plant *Azadirachta indica* obtained from Anand which might be the difference in geographical location or other constituents in the plant. It can hence be said that these plants and the compounds that they contain may have potential anti-diabetic properties and can be used for making drugs for curing diabetes of natural sources in near future.

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