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STUDY OF NUTRATIVE VALUE OF LEPTADENIA PYROTECHNICA AND WITHANIA SOMNIFERA IN BIKANER REGION

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ABSTRACT

It has been discovered what the nutritional content and elemental makeup of the various portions of the plants Withania somnifera and Leptadenia pyrotechnica are that are growing in the region of Bikaner. The research showed that the variety of macro, micro, and proximal components differed not only between plants growing in various locations but also across different portions of the same plant. This was shown to be the case. They carried out biochemical analysis to determine the levels of their major metabolites (carbohydrates, lipids, proteins, and phenols), as well as their antioxidant activity. According to the findings of the current research, L. pyrotechnica and Withania somnifera are both excellent sources of protein, fat, and carbohydrate respectively. Protein-energy malnourishment is a condition that affects children when they do not get an adequate quantity of protein from the food they eat. Because L. pyrotechnica and Withania somnifera are so readily accessible in rural areas, treating malnutrition in developing nations, where the condition affects a disproportionately high number of the population, may be accomplished with the use of both of these plants. Both of the plants that were examined exhibited a considerable level of antioxidant activity. Therefore, it is possible to draw the conclusion that both plants are useful in treating a variety of illnesses that are caused by free radicals generated in the body as a result of intense oxidative stress, and as a result, future study will offer an additional source of antioxidant agents.

Introduction

The majority of ayurvedic medication is derived from plants, similar to how conventional labs produce a vast quantity of chemicals, and plants are the only source of chemical compounds that can be found in nature. The medicinal use of plants and herbs is very significant to the human population.[1] Plants are used all around the globe in the treatment of a broad range of ailments.

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Leptadenia pyrotechnica (Forsk) In India and Pakistan, the Asclepiadaceae family, of which Decne is a member, is known colloquially as the Khimp family.[2] In most cases, it is used as an anti-inflammatory agent, anabolic agent, analgesic agent, astringent agent, and laxative agent. This plant may also be used to treat diabetes as well as eczema. Primary metabolites are synthesised during photosynthesis, and the production of these bioactive molecules is essential to the continued existence, growth, and development of plants. Numerous primary metabolites are used in the production of pharmaceutical substances as pharmacologically active metabolites. The human body is capable of producing a wide variety of oxidant and antioxidant species as a result of its many kinds of metabolic processes. The terms "Reactive Oxygen Species" (ROS) refer to substances such as "super oxide anion," "hydrogen peroxide," and "hydroxyl radicals."[3] Oxidative stress is produced if there is a greater concentration of oxidants in the human body in comparison to antioxidants. Because of this oxidative stress, the body acquired a wide variety of illnesses and conditions, including ageing, cataract cancer, autoimmune disorders, arthritis, cardiovascular and neurological diseases. Some of these conditions and diseases are listed below.

Ashwagandha, also known as Indian ginseng and winter cherry, is a kind of Withania somnifera (Linn.) Dunal. This plant is considered to be one of the most significant substances in Ayurveda and other traditional medical systems. There are 23 different species that make up the genus Withania, which is classified under the Solanaceae family. Withania somnifera and Withania coagulans (Linn.) Dunal are the only two species that have been documented from India. India is home to a total of 23 species.[4] The Withania Linn. Genus may be found in the areas to the east of the Mediterranean as well as in South Asia. The drier regions of India, Africa, and the ancient world are where you'll find the Withania somnifera plant naturally occurring. In the Indian states of Madya Pradesh, Gujarat, and even certain areas of Rajasthan, it is grown on a considerable scale as a crop for commercial purposes. In the Punjab area, the plant known as Withania coagulans is grown for commercial purposes. It is well recognised that Withania somnifera is one of the most effective herbs for pacifying "vata" qualities, and it has also been stated that the plant has adaptogenic action, as well as antitumor, anti- conversant, immunomodelatory, anti-

oxidative, and neurological benefits, in addition to being utilised for nutritional reasons.[5] In order to meet their energy needs, human beings rely on plants to provide them with a variety of complex organic materials. These needs include carbs, lipids, and proteins. The amount of efforts that have been put forward to use medicinal plants as sources of food and energy is significant. In light of this, efforts are undertaken to reduce the number of phytochemical variants and to maintain the compositional homogeneity of herbal products within the confines of stringent regulatory frameworks such as the Dietary Supplements and Health Education Act and the New Natural Health Product Regulations of 2003.[6] A lot of workers attempted to evaluate the nutritional content and mineral composition of therapeutic plants, which are also being utilised as dietary supplements. These plants are employed in a variety of ways.

Materials and methods

Leptadenia pyrotechnica

Collection of plant material

The powdered parts of Leptadenia pyrotechnica was collected from the field of Bikaner.

Evaluation of Primary Metabolites

Plant parts of L.pyrotechnica were harvested and cleaned again and again 4-5 times with water and then put the sample in shade for drying. When samples were dried, grinded in powdered form to conduct quantification of primary metabolites.

Carbohydrates

Total Soluble Sugars

The grinded plant parts (50 mg each) were crushed with 20 mL of 80% ethanol and kept for 24 h. Each sample was centrifuged at 1200 rpm for 15 minutes; the supernatants were collected independently. Distilled water was added to rise volume till 50 mL and processed as per described method.

Starch

The remaining mass obtained after centrifugation was suspended in 5 mL of 52% perchloric acid. Later, 6.5 mL of water was added and shaken dynamically for 5

minutes. The protocol further was done using the phenol sulphuric acid method. A standard regression curve of glucose was used as reference compound. A stock solution of glucose (100µg mL^{-1}) was prepared. Further , 0.1 to 0.8 mL was pipette out and volume was made up to 1 mL with distilled water. Further, 1 mL of 5% phenol was added and shaken gently. 5 mL of conc. sulphuric was added rapidly. Finally the mixture was stable at 26- 30^0C for 20 minutes. The characteristics yellow orange colour was developed. The optical density was measured at 490 nm (Carl Zeiss, Jena DDR, VSU 2 P), against a blank (distilled water). Standard regression curve was computed between the known concentration of glucose and their respective optical density, which followed Lambert Beer's Law.[7]

Proteins

The plant material (50mg each) were crushed in 10 mL of cold 10% trichloroacetic acid (TCA) for 30 min and kept at 4^0C for 24 hours. These mixtures were centrifuged and residues was again suspended in 10 mL of 5% TCA and heated at 80^0 for 30 minutes. The samples were cooled, centrifuged and supernatants of each were discarded. The residue was then washed with distilled water, dissolved in 10 ml of 1N NaOH, and left overnight. Established protocol was used for quantification. A stock solution of BSA (Sigma Chem. Co., St. Louis, USA) was prepared in 1N NaOH (1mgL^{-1}). Different stock (ranging from 0.1 to 0.8 mgL^{-1}) were used and volume of each sample was made to1 mL. To each, 5 mL of freshly prepared alkaline solution (Prepared by mixing 50 mL of 2% Na_2CO_3 in 0.1 N NaOH and 1 mL of 0.5 % $CuSO_4$.$5H_2O$ in 1% Sodium potassium tartarate) was added and kept at room temperature for 10 minutes. In each sample 0.5mL of Folin-Ciocalteau reagent was added rapidly with immediate mixing and optical density of each sample was measured after 30 minutes at 750 nm. Average value was plotted against their respective concentrations to compute regression curve.[8]

Lipids

The dried plant samples were powdered and 100mg was crushed with 10 mL distilled water then transferred to a flask which containing 30 mL of chloroform and methanol[15]. Later, 20 mL of chloroform mixed with 2 mL of water were added and centrifuged. After centrifugation layers were separated, the lower layer of chloroform, which contained all the

lipids, was separated in the preweighed glass vials and the upper aqueous layer of methanol which contained all the water soluble substances and thick interface layer were discarded in each test sample. The chloroform layers which contained all the lipids, dried in vacuo and weighed. Each procedure was repeated three times and their mean values were calculated.

Phenols

The deproteinized plant samples (200mg each) were homogenized with 10 mL of 80% ethanol for 2 hours, and left overnight. The mixtures were centrifuged and the supernatants were collected separately and maintained up to 40 mL by adding 80% ethanol .

Total amount of phenol was done using established protocol. Tannic acid was used as reference. A stock solution of tannic acid was prepared by mixing 40 mg of in 1 mL of 80%ethanol. Different concentrations ranging from 0.1 to 0.8 mL were prepared in the test tube and volume was raised to 1mL by addition of 80% ethanol. To each test tube , 1mL of Folin-Ciocalteau reagent (commercially available reagent was diluted by distilled water in 1:2 ratio just before use) and 2 mL of 20% sodium carbonate solution was added and then mixture was shaken thoroughly. The samples were heated for 1 min and cooled under running water. These reaction mixtures were diluted to 25 mL by adding distilled water and optical density was read at 750 nm against a blank. The optical density of each sample was plotted against the respective concentration of total phenols to compute regression curve.

Methodology for Withania somnifera

The whole plants were collected from the above localities and the entire plants were washed with water and dried in shade. The different parts of the plants were separated (fruit, stem, root, and leaf). The dried plant parts were grind to powder. The powder was used for the determination of mineral composition and nutritive values. However the separated plant material were used to determine the moisture contents as outlined below.

Preparation of plant samples for mineral analysis

One gram of powdered dried plant material was taken in Kjeldhal flask, 25 mL of concentrated H_2SO_4 was added and digestion was carried out on a low flame initially for 10 to 15 min until frothing stops. The digestion at high temp was carried out for 1 to 1½ hours or till the contents of Kjeldhal flask become clear, then the flask was cooled and

content was transferred quantitatively to 100 mL volumetric flask and the final volume was adjusted to 100 mL by adding distilled water. The solution was used for determination of mineral elements through the atomic absorption spectroscopy (AAS) and the flame photometry (FPM). Standard solution of each element was prepared and calibration curves were drawn for each element using AAS/FPM.[9]

Determination of nutritive value

For the determination of nutritive value, the various parameters were estimated using the crushed plant material.

Determination of ash content

10 g of each sample was weighed in a silica crucible. The crucible was heated first over a low flame till all the material was completely charred, followed by heating in a furnace for about 3-5 h at 600 ^{o}C. It was cooled in a desiccator and weighed to ensure the completion of ashing. To ensure completion of ashing it was heated again in the furnace for half an hour, cooled and weighed. It was repeated till the weight become constant (ash become white or grayish white). Weight of the ash gave the ash content.

Determination of moisture content

The samples materials were taken in a flat bottom dish and kept overnight in a hot air oven at 100-110 ^{o}C and weighed. The loss in weight was regarded as a measure of moisture content.

Determination of crude fat

Crude fat was determined by extracting 2 g moisture free samples with petroleum ether in a soxhlet extractor, heating the flask on sand bath for about 6 h till a drop taken from the drippings left no greasy stain on the filter paper. After boiling with petroleum ether the residual petroleum ether was filtered using Whatman No. 40 filter paper and the filtrate was evaporated in a pre-weighed beaker. Increase in weight of beaker gave the crude fat.

Determination of crude protein

Crude protein was determined by using Kjeldhal method. One gram of powdered dried plant material was taken in Kjeldhal flask, 25 mL of diacid mixture was added. The digestion was carried out on low flame initial for 10 to 15 minutes until frothing stops. Then digestion at 1 to 1½ h or till the content in Kjeldal flask become clear the flask was cooled and the contents was transferred quantitatively to the 100 mL volumetric flask and final volume was adjusted to 100 mL by adding distilled water, 10 mL of diluted acid

digested samples was taken in a micro Kjeldhal distillation assembly. The boric acid mixed indicator solution was kept ready at the receiving end to trap ammonia, 30 mL of 40% NaOH was added and distillation was carried out till the colour of the mixture changes and was further continued for some time to trap the all ammonia released. No changes in colour of the red litmus paper indicate the completion of distillation. The quantity of ammonia distilled was estimated by titrating against 0.01N H_2SO_4 or HCl till the colour changes to purple. Determination of crude fibre.

The estimation was based on treating the moisture and fat free material with 1.25% dilute acid, then with 1.25% alkali, thus initiating the gastric and intestinal action in the process of digestion. Then 2 g of moisture and fat free material was treated with 200 mL of 1.25% H_2SO_4. After filtration and washing, the residue was treated with 1.25% NaOH. It was filtered, washed with hot water and then 1% HNO_3 and again with hot water. The residue was ignited and the ash was weighed. Loss in weight gave the weight of crude fibre.

Percentage of carbohydrate was calculated by using the formula, 100-(Percentage of ash +Percentage of moisture + Percentage of fat + Percentage of protein).[10]

Nutritive value

Nutritive value was finally determined by:

Nutritive value = 4 x Percentage of protein + 9 x Percentage of fat + 4 x Percentage of carbohydrate.

Results and discussion

Quantification of Primary metabolites: The present investigations quantify that L. pyrotechnica contain many primary metabolites like carbohydrates, proteins, phenols and lipids. In the present investigation, various plant parts were estimated for their primary metabolites viz, total soluble sugar, starch, lipid, protein and phenol

Proteins: The maximum amount of protein was observed in shoot of L.pyrotechnica (14±11.22mg/gdw). High level of protein indicate their food value.

Lipid: The observed quantity for lipid was higher in shoot of L.pyrotechnica (141±0.95mg/gdw) while minimum in root of L.pyrotechnica (21±0.34mg/gdw).

Phenol: The amount of phenol was highest in root of L.pyrotechnica(0.23±0.004mg/gdw)

Carbohydrate: Total soluble sugar: in the present study, among all the samples, shoot of L.pyrotechnica exhibited higher total soluble sugar level (5.6±0.23mg/gdw)

Starch: The maximum content of starch was observed in root of L.pyrotechnica (4.1±0.17mg/gdw) and minimum in shoot of L.pyrotechnica (1.1±0.06mg/gdw).

Withania somnifera

The results of the macro elements, the micro elements, the components of nutritional value, and the nutritive value themselves are observed. The measurements of the proportion of nitrogen in Bikaner range from 0.08 to 1.75, depending on whether the sample was taken from the root or the fruit. The nitrogen content of the stem and leaf samples was measured and found to be modest at 0.89% and 0.86%, respectively. The lowest rates of nitrogen were found in the root samples from Bikaner, which came in at 0.08%. The highest levels of nitrogen were found in the fruits samples from Bikaner, which came in at 1.77%. Nitrogen is an element that is necessary for the formation of structural proteins. Purines, pyrimidines, porphyrins, and coenzymes are all good places to look for it.[11] When nitrogen is provided to the plant in excess, the plant develops leaves that are a dark green colour and has an abundance of foliage. However, the plant's root system grows more slowly as a consequence, and the plant displays a high shoot to root ratio.[12]
According to the findings of the current research, the nitrogen content of the fruit samples was the greatest, and the researchers believe that this is related to the buildup of nitrogen in the preserved items. An investigation was conducted into the ways in which the quantity of total nitrogen changed in the root, stem, leaf, and seeds of a wide bean plant from the seedling stage until maturity. According to the findings of the investigation, the greatest percentage of nitrogen was recorded in the seed samples.[12] The current observation confirms the findings of the inquiry described above. The proportion of phosphorus in the Bikaner samples varied from 0.13 to 0.45, and the lowest amount of phosphorus in the roots samples was either 0.13% or 0.14%, depending on which sample you looked at. The leaf samples from Bikaner were found to have the greatest percentage of phosphorus.

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Phosphorus is readily transported from one organ to another in most plant species. Phosphorus from older leaves accumulates in younger leaves as well as in flowers and seeds that are still growing. [12]

Phosphorus is an important component of several sugars that play a role in metabolic activities like as photosynthesis, respiration, and others. In addition to this, it is a component of nucleotides, such as those found in RNA and DNA, as well as the phospholipids that are found in membranes 13. At Bikaner, the amount of sodium comprised somewhere between 0.41 and 0.55 of the total. The greatest sodium concentrations found in the leaf samples came in at 0.55% and 0.77% respectively. A further conclusion that can be drawn from these findings is that the margin of error between the highest and lowest % salt levels found in the various plant components was quite small. Allen and Arnon[13] confirmed that a number of blue-green algae and higher plants had a demand for salt in their diets. It has been suggested that sodium may partly replace potassium in both higher and lower plant species.[14,15]

The potassium content ranged anywhere from 1.74 to 3.81 percent at Bikaner and 0.88 percent overall. The fruit samples from Bikaner had potassium levels that were at least 1.74 percent lower than the minimal amount. The leaf samples were found to have the greatest level of potassium, and the results for the Bikaner samples showed that it was 3.81%. In addition to nitrogen, phosphorus, and potassium from commercial fertiliser, potassium was also applied. As is the case with nitrogen and phosphorus, it is simple for K+ to move from more mature organs to younger ones; hence, indications of K+ depletion develop first on older leaves. The current findings are in agreement with those from earlier studies, which found that potassium acts as an activator of several enzymes that are necessary for photosynthesis and respiration. Furthermore, potassium stimulates enzymes that are required for the formation of starch and proteins.[12]

The stem samples from Bikaner had a higher proportion of calcium than the other samples (1.24%). The lowest proportion of calcium was found in the root samples from Bikaner, which clocked in at 0.158 percent. The magnesium content was greatest in the leaf samples from each of the locations, with Bikaner having 0.32% of the total magnesium content. The fruit samples from Bikaner were found to have magnesium levels at or above the minimal limit of 0.08%. The leaf samples from Bikaner had the greatest iron content (741.0 ppm), which was measured in parts per million. The nutritional value of the various

plant components found in the various locations may be determined after having their percentages of moisture, ash, crude protein, carbohydrate, crude fibre, and crude fat assessed.

Conclusion

According to the findings of the current research, L. pyrotechnica and Withania somnifera are both excellent sources of protein, fat, and carbohydrate respectively. Protein-energy malnourishment is a condition that affects children when they do not get an adequate quantity of protein from the food they eat. Because L. pyrotechnica and Withania somnifera are so readily accessible in rural areas, treating malnutrition in developing nations, where the condition affects a disproportionately high number of the population, may be accomplished with the use of both of these plants. Both of the plants that were examined exhibited a considerable level of antioxidant activity. Therefore, it is possible to draw the conclusion that both plants are useful in treating a variety of illnesses that are caused by free radicals generated in the body as a result of intense oxidative stress, and as a result, future study will offer an additional source of antioxidant agents.

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