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Synthesis and Assessment of Antioxidant Capacity of Hypoglycaemic Plants

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Abstract - Natural products are critical to health-care systems. The numerous sources of herbal substances and plants have served as the foundation for chemical compounds utilised as input materials in pharmaceutical formulations such as synthetic medications. Multiple approaches were employed to assess the antioxidant activity of the selected plant extracts. The goal of this research is to assess the characteristics and treatment of diabetes patients in our country. The study emphasises the necessity and attention on medicinal herbal plants in order to separate the bioactive components and make their hypoglycaemic effects evident. Diabetes was treated with a drug made from medicinal herbs. The biological effects of these chemicals are discovered, which facilitates in the conversion of these substances into medications or lead molecules for further drug development. As a result, the goal was to characterise the active components recovered from the strong extract using chromatography. It provides the community with effective, cheap therapy while increasing public trust in alternative medicines.

Keywords - Antioxidant Properties, Hypoglycaemic Plants, Diabetes, Herbal Drugs.

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INTRODUCTION

Humans have long relied on plants to provide their fundamental needs, including food, clothing, shelter, transportation, fertilisers, flavours, and smells, as well as medicines. The herbs gave rise to a traditional medicinal system that has lasted more than a century in countries such as China and India [1]. Eighty percent of the world's population still relies on traditional medicine systems for their healthcare needs, and these plant-based systems continue to play an important role in health care. Natural plant products have a significant role in the health care system for 20% of the population, particularly in residential nations. These drugs are obtained from a range of sources, including terrestrial plants, terrestrial microorganisms, terrestrial sea species, terrestrial vertebrates, and terrestrial invertebrates [2].

Indian System of Medicine (Ayurveda)

Ayurveda, the world's oldest medical system, is said to have existed for at least 5,000 years. According to Ayurveda, all matter in the universe is made up of air, space, energy, liquid, and solid. Homeostasis is exhibited by a variety of biological components or stable quantitative indicators that depict the body in a healthy state. As a result, the idea is critical for maintaining the internal environment, buffer system (blood and tissue fluids), and homeostasis of the organism [3].

Standardization of Natural Drugs

Drugs are distributed in original or traditional systems as polar solvent extracts. The frequency of a fresh natural plant fraction or powdered crude medicine is slightly less than that of a rule. Therefore, components of medicinal plants should be dependable and devoid of harmful substances like heavy metals, pesticides, infectious diseases, radioactive pollution, etc. The natural plant is concentrated on a specific solvent extraction again and over again, or water decoction, or as stated in the oldest scriptures [4]. The extract has to be tested for any natural activity found in an animal model used for research. The bioactive material extract should match fingerprints in terms of the main complex or active principle. Clinical investigations are demonstrating the potential benefits for further scientific application. Under no circumstances can the herbal medications created be sold as nutraceuticals or health foods, but rather as prescription or over-the-counter medications based on pathological consideration [5].

The most prevalent kind of diabetes mellitus is type 2, and its two most prominent features are insulin resistance and insulin secretion. There aren't many helpful, highly precise markers for type 2 diabetes, but the existence of risk factors like obesity increases the probability of getting the disease. These amounts are insufficient to sustain normoglycemia in the context of insulin resistance.

This relative insulin insufficiency distinguishes between patients with normoglycemia and diabetes who are insulin resistant [6]. The bulk of the genes linked to type 2 diabetes, as opposed to insulin resistance, are involved in insulin production. These people do not need insulin therapy to survive despite having type 2 diabetes at birth and frequently during their lifetime. However, the risk of growing macro and micro vascular complications is increased in such patients. Obesity itself results in insulin resistance, which is a type 2 diabetes complication. Many people who are not considered obese by conventional standards, such as body mass index, may have an improved proportion of body fat that is mostly distributed in the abdomen region. Type 2 diabetic ketoacidosis frequently develops in conjunction with the stress of another condition, such as an infection [7]. The extreme hyperglycaemia and ketoacidosis of type 2 diabetes necessitate prompt insulin treatment. Age, obesity, and a lack of exercise all increase the risk of type 2 diabetes. Women with past GDM, those with hypertension, or those with dyslipidemia have had it more frequently. Although type 2 diabetes is certainly genetically predisposed, it is connected with strong families. Without meeting the criteria for the diagnosis of diabetes, the disease process might result in varying degrees of impaired glucose metabolism, such as impaired fasting glycemia and impaired glucose tolerance. In order to attain acceptable glycaemic control in type 2 diabetes, weight loss, exercise, and oral hypoglycaemic medication are recommended [8].

Non-enzymatic enzymes' antioxidant abilities are reasonable for reactive species with harmful properties. These defences are crucial because they completely keep out free radicals, giving biological places the maximum level of security. It makes use of SOD, catalase, and other specialised enzymatic antioxidants. Among the non-enzymatic antioxidants employed are vitamins C and E, carotenoids, thiol antioxidants, lipoic acid, natural flavonoids, and a hormone supplement [9]. Biological systems offer an internal environment that supports survival, development, and reproduction in addition to the external environment. However, oxidative damage invaded cellular regions, impairing their organisation and function, which was the conundrum of aerobic existence. The oxygenic hazard is being caused by antioxidants, which developed along with our oxygen-rich atmosphere. The nutritional antioxidants, which are mostly derived from herbs, are believed to have a significant role in managing diabetes. The study of naturally occurring hypoglycaemic compounds with antioxidant activity thus assumes paramount significance [10].

A multidisciplinary approach is required since the development process from the plant to a purified, pharmacologically active component is highly laborious and lengthy. For the inquiry to be successful in the long run, the collecting of plant species is essential. Even yet, focused collection is provided by random sampling for chemotaxonomic associations and ethno medical in order to be obtained from conventional medicine. To identify and authenticate plants for use in industry and for future study, phytochemical analysis will be a beneficial tool. So, many of the plants that were chosen based on their ethnomedical usage had phytochemical testing done on them [11]. Antioxidant activity of a studied substance is correlated with total phenol concentration. Antioxidants are becoming more popular as preventative and helpful agents that hunt down free radicals and stop the harm caused by them. Numerous compounds, including vitamins, flavonoids, terpenoids, carotenoids, tannins, and phytoestrogens are responsible for these qualities. It is advised to consume plants with high antioxidant potential such as *Curcuma longa, Capsicum frutescens, Zinziber officinale, Allium cepa, and Schisangra chinesis*. Experimental evidence supports the use of many antioxidants of plant origin as powerful preventative measures against free radical-mediated harm [12].

In order to test the antioxidant activity of the chosen plant extracts, multiple techniques were used. The purpose of this study is to evaluate the characteristics and treatment of diabetic patients in our nation. In order to distinguish the bioactive compounds and make their hypoglycaemic effects clear, the study demonstrates the importance and focus on medicinal herbal plants. A medication derived from medicinal plants was crucial in the treatment of diabetes. The primary groups of anti-diabetic herbs now used in traditional medicine include *Allium cepa, Allium sativam, Aloe vera, Azadirachta indica, Gymnema sylvestro, Syzygium cumini, Pterocarpus marsupium*, etc [13]. Therefore, the potent fraction of the compound was tested for in vivo antidiabetic effect in high fat induced diabetes carrying rats in the current investigation. Additionally, research on fractions' in vitro anti-diabetic and diabetes-related wound-healing activities were conducted. Plant research benefits greatly from the isolation of phytoconstituents from the active extracts. These components might be used as benchmark compounds to standardise them. The biological actions are identified, which aids in turning these substances into medicines or lead molecules for additional drug development. Therefore, the objective was to characterise the active components isolated using chromatography from the powerful extract. It gives the community access to effective, affordable treatment while fostering public trust in alternative therapies [14].

METHODOLOGY

- **Collection of Plant Materials:** *Barleria noctiflora* was collected during the winter season.
- **Pharmacological Studies:** The various pharmacological studies shall be adopted for the current study. These include microscopical studies, T.S of a leaf and stem followed by their sectioning, their powder analysis, forming photomicrograph, performing quantitative microscopy of Leaf Constants, measuring

Stomatal index and Stomatal number, vein inlet and vein termination number and finding *C. palisade* ratio.

- **Estimation of Total Phenol Content:** The Folin-Ciocalteu technique was used to determine the total phenol concentration of the extracts.
- **Estimation of Total Flavonoid Content:** The aluminium chloride utilised in the colorimetric technique will be used to determine the total flavonoid content. At 415 nm, the absorbance will be measured using a spectrophotometer.
- ***Compound Antioxidant Investigations in Vitro*:** In vitro antioxidant tests will be carried out to compare the antioxidant activity of the plant extracts. For in vivo experiments, the extracts with the best antioxidant properties will be chosen. Following the steps outlined below, the various plant extracts' scavenging abilities against various radicals will be tested. The absorbance will be measured against a blank solution in each experiment. A control shall also be carried out without extract or standard addition.
- ***In-Vitro* and *In-Vivo* Anti-Diabetic Studies:** The research on in vitro antioxidant activity shall reveal effective ethanol extracts that were chosen and fractionated with various solvents from ethyl acetate and n-butanol. These extracts will then be examined for in vitro antidiabetic activity by inhibiting -amylase and -glucosidase. The percentage of enzyme inhibition need to inhibit an enzyme by 50% which will be used to express the results, which will be given in IC50.
- **Pharmacological Screening of *Barleria noctiflora*:** *Barleria noctiflora's* ethyl acetate fraction (EAFBN) shall show strong anti-diabetic effect in *in vitro* experiments. Therefore, for an *in vivo* assessment of acute toxicity, subacute toxicity, anti-diabetic efficacy, and wound healing activities, we shall choose the ethyl acetate fraction of *Barleria noctiflora* (EAFBN).
- **Histopathological Studies of Pancreatic Tissues:** Rat pancreatic tissues will be extracted and utilised for histopathological research. Each group's tissue will be divided into pieces measuring 1cm, fixed in a 10% formaldehyde solution, dehydrated in ethanol at progressively higher concentrations, cleaned in xylene, and then will be encased in paraffin.
- **Statistical Analysis:** Using the in Stat v. 2.02 programme, all results shall be reported as mean Standard Error of Mean (SEM) and will be examined for significance using an ANOVA. Groups will then be compared using the Dunnett test (Graph Pad Software Inc.). At the P0.05 level, differences between groups (p Value) will be deemed significant.
- **Isolation and Characterization of Phytoconstituents:** The process of column chromatography is frequently used to separate, isolate, and purify natural materials. By using column chromatography, the separation may be accomplished by altering the polarity of the mobile phase. Different analytical methods, including Ultra Violet (UV), Infrared (IR), Nuclear Magnetic Resonance (NMR), and Mass Spectroscopy, can be used to characterise the separated chemicals (MS). To separate the phytoconstituents from the ethyl acetate fraction, column chromatography was used.

RESULTS

- The majority of current plant-based drug discovery relies on bioactivity-guided isolation techniques; using natural products has shown to be the most effective way for finding novel drugs and leads. Untapped chemical resources abound in plants. Compared to bacteria, plants have a far wider variety of chemical compounds.
- The findings of the standardisation might provide a lot of light on the botanical identification of the *Barleria noctiflora* leaves and stem. This might serve as a foundation for determining the plant's legitimacy and for separating the medicine from its adulterants and other species. The appropriate crude medicine was found by looking at the macroscopic features.
- The *Barleria noctiflora* powder had a noxious odour and tasted slightly sour and sweet. It was a dark greenish grey tint. The powder revealed many unicellular, slightly curved covering trichomes under microscopic analysis (table 1).

Table 1: Data for extractive values and loss on drying for powdered of *Barleria noctiflora*

Analytical parameters	Percentage (%w/w)
Alcohol soluble extractive	7.36
Water soluble extractive	8.24
Loss on drying	2.67

- The ash values were significant since ash may come from both the plant and outside sources, including sand and dirt that adhere to the drug's surface. The ash values changed in many cases of crude pharmaceuticals, and studying them might provide insight on the substance's quality and purity (table 2).

Table 2: Data for ash values for powdered of *Barleria noctiflora*

Ashes	Ash values (%w/w)
Total ash	13.5
Water soluble ash	9
Acid insoluble ash	2

- The findings imply that the powdered has a high extractive value that is water soluble. Since moisture makes it easier for

microorganisms to thrive or for enzymes to break down, which causes degradation, the amount of moisture in the medicine is revealed by the amount of loss on drying. The identification and standardisation of unprocessed medicines are the quantitative microscopy parameters.

- Flavonoids, alkaloids, phenols, saponins, steroids, glycosides, tannins, protein, and carbohydrates were all found in the plant extracts used in the phytochemical research. For the aim of evaluating crude medicines, total phenol and flavonoids are crucial (table 3).

Table 3: Preliminary phytochemical screening of *Barleria noctiflora*

Chemical constituents	Water extract	Alcohol extract	Chloroform extract	Petroleum ether extract
Flavonides	+	+	-	-
Alkaloids	+	+	-	-
Phenols	+	+	-	-
Saponins	+	+	-	-
Steroids	-	-	+	+
Glycosides	+	+	-	-
Tannins	+	+	-	-
Protein	+	+	-	-
Carbohydrate	+	+	-	-

- The findings found for the extracts in the DPPH technique indicated that extracts were probably capable of scavenging free radicals.

- Studies on the inhibition of -amylase and -glucosidase in vitro showed that EAFBN had an inhibitory effect on intestinal digestive enzymes.

- Numerous studies have shown that elevated blood triglycerides, low HDL cholesterol, and elevated LDL cholesterol levels often coexisting are major predictors of coronary heart disease (CHD). In individuals with hypertriglyceridemia, the postprandial metabolism of lipids is particularly important. It has been demonstrated that it contributes to atherogenesis and causes endothelial dysfunction. HDL cholesterol level was dramatically raised as a result of the large reductions in total cholesterol, triglyceride, and LDL cholesterol levels. Therefore, the EAFBN fraction demonstrated prevention and decreased lipid-induced disorders such diabetes, atherogenesis, and CHD risk factors (figure 1).

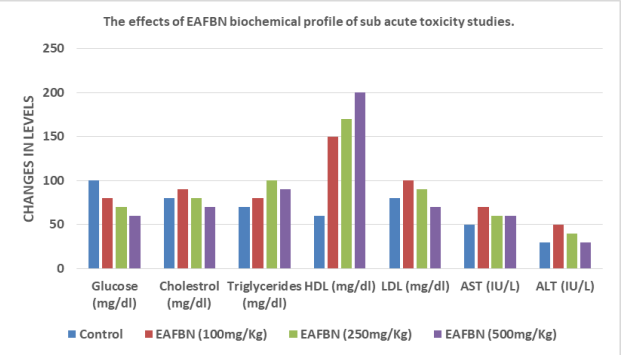

Figure 1: Effects of EAFBN biochemical profile of sub-acute toxicity studies

- Increased glucose levels have a toxic impact on -cells according to the notion of glucose toxicity, which describes persistent exposure to abnormalities and high amounts of glucose as a pathogenic force. As a result, all dosages were significantly reduced in EAFBN-treated mice (figure 2).

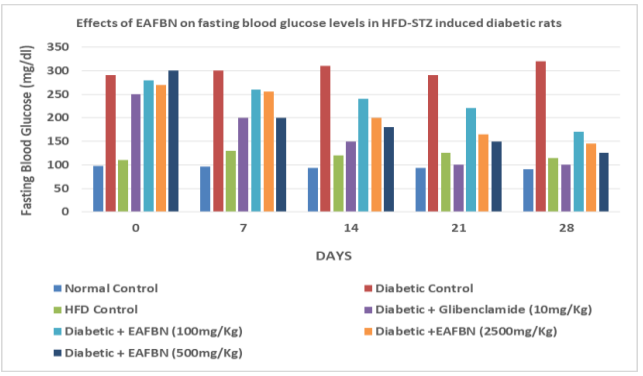

Figure 2: Effects of EAFBN on fasting blood glucose levels in HFD-STZ induced diabetic rats

CONCLUSION

The analysis which accompanies illustrates how antioxidants are becoming more and more popular as helpful and defensive substances that scavenge free radicals and mitigate the damage they cause. Numerous compounds, including vitamins, flavonoids, terpenoids, carotenoids, tannins, and phytoestrogens are responsible for these qualities. Consuming plants like *Schisangra chinesis, Curcuma longa, Capsicum frutescens, Zinziber officinale, and Allium cepa* that have great antioxidant potential is advised. According to experimental results, a variety of antioxidants with plant origins can be utilised as powerful inhibitors of free radical damage. Strong and efficient herbal medicines must undergo traditional scientific scrutiny before being approved for use in the treatment of illnesses. In Indian traditional medicine, *Barleria noctiflora* is said to provide medicinal advantages. Existing patent laws have made it necessary to protect the claims of these tried-and-true folk

remedies. It is crucial to start cataloguing the parts and purposes of these therapeutic plants as a result.

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