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Phytochemical Evaluation, Chromatographic Screening,Biological Evaluation of Antioxidant and Antidiabetic activity of Sesbania grandiflora

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Abstract

A vegetable is the classification that is given to the species Sesbania grandiflora, which belongs to the family Fabaceae and is consumed on a regular basis by people in Indonesia. Studies have demonstrated that the secondary metabolites that are found in these plants have the ability to perform a variety of biological functions, including anti-cancer, anti-cancer, anti-bacterial, and anti-diabetic properties. In order to accomplish the objectives of this study, the stembark of Sesbania grandiflora was subjected to a phytochemical analysis by means of Thin Layer Chromatography (TLC), and the results of such an analysis were presented.These compounds are often associated with significant biological activities and contribute to the therapeutic potential of the plants. Total phenolic content was quantitatively assessed in the methanolic extracts, revealing values of 50.72% w/w for *Sesbania grandiflora*. Phenolic compounds are known for their antioxidant properties, which are largely influenced by their molecular structure, the availability of phenolic hydrogens, and their ability to stabilize the resulting phenoxyl radicals through hydrogen donationwhich is implicated in various chronic diseases.This antioxidant potential is significant, as reactive oxygen species (ROS) contribute to cellular damage and inflammation, linking oxidative stress to a range of pathological conditions.The potential antidiabetic effects of *Sesbania grandiflora* were evaluated in alloxan-induced diabetic models. This effect is likely due to multiple mechanisms, including the enhancement of peripheral glucose utilization and reduction of intestinal glucose absorption. the findings highlight the potential of these plant extracts in managing diabetes, particularly in populations where traditional remedies are preferred.

Keywords: Antioxidant activity,Hyperglycemia,Glibenclamide,Alloxan, Natural productsect.

1. Introduction

Natural products have been utilized by humans for centuries, serving as a foundation for traditional medicine, food, and various industrial applications. This document explores the significance of natural products, their diverse applications, and the ongoing research that highlights their potential benefits in modern society.[1] From pharmaceuticals derived from plants to the use of natural ingredients in cosmetics and food, the relevance of natural products continues to grow in an era increasingly focused on sustainability and holistic health.Natural products are chemical compounds or substances produced by living organisms. They are primarily derived from plants, animals, and microorganisms and have been integral to human life, providing essential resources for health, nutrition, and industry.[2] The exploration of natural products has led to significant advancements in medicine, agriculture, and environmental sustainability.

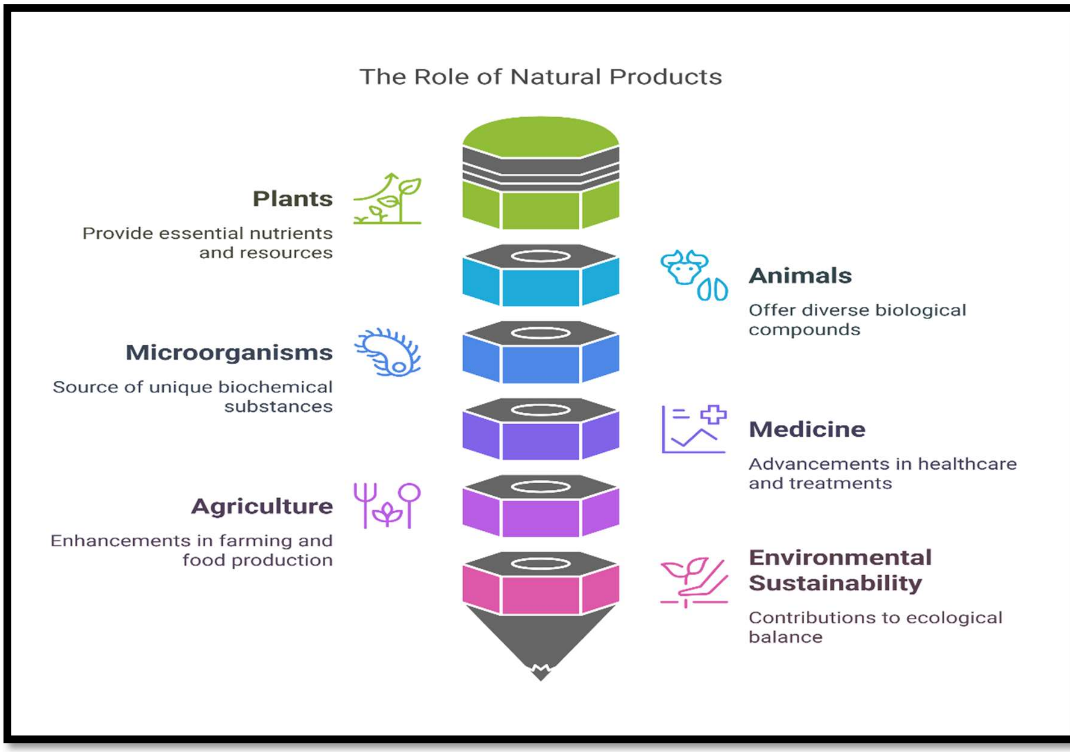


Fig.No.1: Flow diagram represent the role of natural products

Throughout history, plants have remained a cornerstone for medicinal compounds crucial to human health. India boasts an exceptional diversity of medicinal flora, with plants serving as therapeutic agents for various ailments and holding promise for the development of new pharmacological treatments.[3] Despite the pharmaceutical industry's advancements in antibiotics, the increasing issue of microbial resistance poses significant challenges, emphasizing the need for plant extracts with alternative mechanisms of action to combat resistant pathogens.[4]The World Health Organization defines traditional medicine as “the sum total of all the knowledge and practices, whether explicable or not, used in diagnosis, prevention, and elimination of physical, mental, or social imbalance, relying exclusively on practical experience and observation passed down through generations.” Thus, traditional systems of medicine can be regarded as dynamic amalgamations of extensive medical knowledge and experiential wisdom.[5].

2. Various applications of Natural Products[6-8]

I. Pharmaceuticals

Many modern medicines are derived from natural products. For instance, the pain reliever aspirin was originally derived from willow bark, and the cancer drug paclitaxel comes from the Pacific yew tree. The study of natural compounds continues to be a rich source for drug discovery, with researchers exploring various plants and organisms for new therapeutic agents.

II. Food and Nutrition

Natural products play a crucial role in our diet. Fruits, vegetables, herbs, and spices not only provide essential nutrients but also contain bioactive compounds that can promote health. For example, antioxidants found in berries and flavonoids in tea have been linked to various health benefits, including reduced inflammation and improved heart health.

III. Cosmetics and Personal Care

The beauty industry has increasingly turned to natural ingredients as consumers seek safer and more environmentally friendly products. Ingredients such as aloe vera, coconut oil, and shea butter are popular in skincare formulations due to their moisturizing and healing properties. Natural products in cosmetics often appeal to consumers looking for clean beauty options.

IV. Agriculture

Natural products are also vital in sustainable agriculture. Biopesticides and biofertilizers derived from natural sources help reduce the reliance on synthetic chemicals, promoting healthier ecosystems. Additionally, plant-based compounds can enhance crop resilience against pests and diseases, contributing to food security.

V. Environmental Sustainability

The use of natural products extends to environmental applications, such as biodegradable materials and renewable energy sources. Research into natural polymers and biofuels derived from organic matter is paving the way for more sustainable practices that minimize environmental impact.

3. Protective Mechanisms Against Oxidative Stress[9-11]

To counteract the adverse effects of ROS, living organisms have developed a complex array of protective mechanisms, including:

Antioxidant Enzymes: Enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase play crucial roles in neutralizing ROS. **Non-Enzymatic Antioxidants**: These include vitamins (C and E), glutathione, and various phytochemicals that scavenge free radicals and reduce oxidative damage.[12]

Antioxidants can function through multiple mechanisms:

Free Radical Scavenging: Directly neutralizing free radicals to prevent them from causing cellular damage.

Metal Chelation: Binding to free catalytic metals (e.g., iron and copper) to prevent them from catalyzing the formation of reactive radicals.

Electron Donation: Acting as electron donors to stabilize free radicals.

Sources of Antioxidants

Natural antioxidants are primarily derived from plant-based foods. The following categories are particularly rich in antioxidant properties:

Fruits and Vegetables: Rich in vitamins (C and E), carotenoids, flavonoids, and phenolic acids. Berries, citrus fruits, and leafy greens are especially noted for their high antioxidant content.

Whole Grains: Sources of tocopherols, phenolic compounds, and other phytochemicals that contribute to overall antioxidant capacity.

Nuts and Seeds: Contain vitamin E, selenium, and various polyphenols that exhibit significant antioxidant effects.

Polyphenols, a major class of plant-derived antioxidants, are known for their dual function as free radical scavengers and metal chelators. Common polyphenolic compounds include flavonoids, phenolic acids, and tannins, which have been extensively studied for their health benefits.[13]

4. Antioxidants in Clinical Research and Application[14-16]

The growing understanding of the role of antioxidants in health has led to increased interest in their potential therapeutic applications.

Cancer Therapy: Research indicates that certain antioxidants may enhance the efficacy of conventional cancer treatments by reducing oxidative stress and protecting normal cells from damage.

Cardiovascular Health: Antioxidants like vitamin E and flavonoids from dietary sources are being studied for their protective effects against heart disease.

Neuroprotection: Antioxidants may play a role in mitigating oxidative damage in neurodegenerative diseases, potentially slowing disease progression.

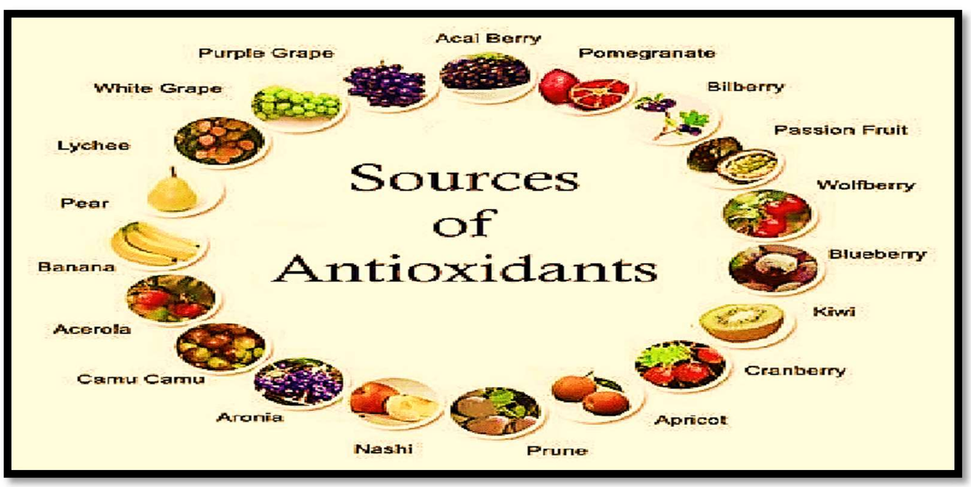


Fig.No.2: Flow chart showing the sources of Antioxidants

5. Diabetes Mellitus: An Overview of a Growing Epidemic[17]

Diabetes mellitus represents a significant public health challenge characterized by chronic illness, substantial morbidity, and mortality. The condition is projected to affect approximately 300 million individuals globally by 2025. The alarming rise in diabetes prevalence can be attributed to multiple factors, including an increasing obesity epidemic, primarily driven by sedentary lifestyles and high-fat dietary patterns, coupled with an aging population.[18] It is noteworthy that over 85% of individuals diagnosed with diabetes mellitus have type 2 diabetes, with the lifetime risk of developing this condition approaching 20% in the United States alone. Data from the Third National Health and Nutrition Examination Survey indicate that the prevalence of type 2 diabetes among adults (ages 20 and older) is approximately 5.1%, with undiagnosed cases at 2.7%, and impaired glucose tolerance (IGT) at 6.9% based on fasting plasma glucose levels.[19] These statistics are particularly concerning given that diabetes is a leading cause of new cases of blindness, end-stage renal disease, and lower extremity amputations. Moreover, nearly half of the diabetes-related mortality can be attributed to cardiovascular diseases, significantly contributing to overall cardiovascular mortality in the United States. The long-term complications associated with diabetes, encompassing both microvascular and macrovascular diseases, have been extensively documented in the literature.[20]

5.2 Types of Diabetes Mellitus[21-23]

Diabetes mellitus can be classified into several categories, with type 1 and type 2 diabetes being the most prevalent. Type 1 diabetes is often referred to as insulin-dependent diabetes mellitus (IDDM), whereas type 2 is classified as non-insulin-dependent diabetes mellitus (NIDDM). The classification of type 1 diabetes is typically based on etiology, with patients exhibiting little to no endogenous insulin secretion requiring insulin therapy for survival. Clinical type 1 diabetes is further divided into two main forms: type 1a and type 1b. Type 1a, accounting for about 90% of type 1 cases in Europe, is believed to result from autoimmune destruction of pancreatic β-cells, leading to insulin deficiency. In contrast, type 1b (idiopathic) encompasses cases without clear autoimmune indicators, representing approximately 10% of type 1 diabetes instances. Type 1a diabetes is characterized by the presence of specific antibodies, such as islet cell antibodies (ICA) and anti-glutamic acid decarboxylase (anti-GAD), which are markers of the autoimmune process targeting β-cell function. Autoimmune disorders, including Graves' disease, Hashimoto’s thyroiditis, and Addison’s disease, may coexist with type 1 diabetes mellitus.[24] Conversely, the etiology of type 1b diabetes remains poorly understood, with some patients experiencing persistent insulin deficiency and a higher susceptibility to ketoacidosis, but without evidence of autoimmune involvement. This form is more frequently observed in individuals of African and Asian descent. Type 2 diabetes is the most common form of diabetes, characterized by defects in insulin secretion and insulin resistance. In Western countries, the prevalence of type 2 diabetes affects up to 7% of the population, while globally, it impacts 5-7% of individuals. However, these figures may be underestimated, as many cases, potentially up to 50% in certain populations, remain undiagnosed. The prevalence of type 2 diabetes varies significantly across regions, with rates as low as less than 1% in rural populations of developing countries, such as rural Melanesians and rural Chinese, to over 50% among the Pima Indians in Arizona. Notably, there is a higher incidence of type 2 diabetes in urban areas compared to rural locales, often correlating with lifestyle transitions from traditional practices to modern "Westernized" living.[25]

Traditionally, type 2 diabetes has been associated with individuals over the age of 40, often linked to obesity, reduced physical activity, and genetic predisposition. However, recent data indicate an alarming trend of rising type 2 diabetes incidence among adolescents and even children. In some regions, the prevalence of childhood type 2 diabetes has surpassed that of type 1 diabetes. Management of the disease typically involves dietary modifications, increased physical activity, and pharmacological interventions utilizing hypoglycemic agents. The intricate relationship between cytotoxicity, the use of traditional plants for medicinal purposes, and the rising prevalence of diabetes mellitus underscores the critical importance of ongoing research in these areas. Understanding the potential toxic effects of plant-derived compounds is essential to ensure safety and efficacy in medicinal applications. Additionally, addressing the diabetes epidemic requires comprehensive strategies that encompass lifestyle changes, public health initiatives, and a better understanding of the disease's underlying mechanisms.

6. Plant profile of Sesbania grandiflora [27]

Sesbania grandiflora, commonly known as the vegetable hummingbird or agati, is another species with notable therapeutic potential. The stem bark is rich in various phytochemicals, including flavonoids and tannins, which are associated with numerous health benefits such as enhancing immune response and exhibiting anti-diabetic effects. The rigorous selection process of these plants was informed by their documented biological activities and the necessity for alternative medicinal resources, especially in the context of increasing antibiotic resistance and the demand for natural therapeutics. By focusing on these specific plants, the study aims to contribute to the understanding of their pharmacological properties and potential applications in modern medicine.[28]

Fig.No.3: Plant profile of Sesbania grandiflora

Description

Growth Habit: A large shrub or small tree that can reach heights of 3-10 meters. It has a bushy appearance with a spreading canopy. **Leaves**: The leaves are pinnate, consisting of numerous small leaflets (usually 10-20 pairs) that are green and glabrous. Leaflets are around 2-6 cm long. **Flowers**: Large, showy flowers are typically white to pale yellow, sometimes with purple markings, and measure about 5-10 cm across. They grow in clusters and attract various pollinators, especially bees. **Fruits**- The fruit is a pod that can be up to 30 cm long, containing several seeds. The pods are initially green and turn brown when mature.[29]

Medicinal Uses[30]

Culinary-The young leaves, flowers, and pods are edible and used in various dishes, particularly in Asian cuisines. They are often cooked in soups or stir-fried. **Medicinal**-Traditionally used in folk medicine for various purposes, including treating skin conditions and digestive issues. **Forage**-The foliage is nutritious and used as fodder for livestock. **Erosion Control**-Due to its fast growth and extensive root system, it is often planted for soil stabilization and erosion control.

7. Results and Discussion

7.1 Extractive value:

The extracts were derived from Sesbania grandiflora, yielding the following percentages: 1.21% for petroleum ether, 2.46% for chloroform, and 13.32% for methanol. Similarly, the extracts from Cayratia trifolia were obtained with the following percentages: 0.89% for petroleum ether, 3.6% for chloroform, and 16.63% for methanol (see Table 1).

Table No. 1 Extractive values (% w/w yield) of plant material with different solvents.

Plant Extract of Sesbania grandiflora	% of extract obtained
	Sesbania grandiflora
Petroleum ether	0.89
Chloroform	3.6
Methanol	16.63

7.2 Phytochemical investigation of pods of sesbania grandiflora

Preliminary phytochemical analysis indicated the presence of various phytochemicals in the extracts of Sesbania grandiflora pods and Cayratia trifolia stem bark, as presented in Table 2. Specifically, Sesbania grandiflora exhibited sterols in the petroleum ether extract, glycosides and flavonoids in the methanol extract, as well as triterpenoids in both the petroleum ether and methanol extracts. Additionally, tests on Sesbania grandiflora extracts confirmed the presence of alkaloids in the chloroform extract. The separation of extract constituents was performed using column chromatography; however, fractions containing inseparable matter and compounds obtained in minimal quantities were discarded.

Table No. 2. Phytochemical analysis of different extracts of stem bark of Sesbaniagrandiflora .

S.No.	Testname	Plant Extracts					
		Sesbania grandiflora					
		Pet ether	Chloroform	Methanol	Pet ether	Chloroform	Methanol

Testfor Sterols	Salkovaski TestLibermant est	+	-	-	+	-	-
Testfor Glycosides	β-naptholtest	-	-	+	-	-	+
Test for Alkaloids	Mayer'stet, Wagner'stest, Hager'stest	-	-	-	-	+	-
Testfor Triterpenoid s	Libermann-Burchardtest	+	+	-	+	-	-
Test for Flavonoi ds	ShinodhaTestF erric chloride test	-	-	+	-	-	+
Testfor Anthraquino nes	Juglonetest	-	-	-	-	-	-
Test for Carotenoi ds	Ferric chloridetest	-	-	-	-	-	-
Testfor Tannins	Carr-pricetest	-	-	+	-	-	+

+ indicate Positive test - indicate Negative test

7.3 TLC of petroleum ether extract of sesbania grandiflora

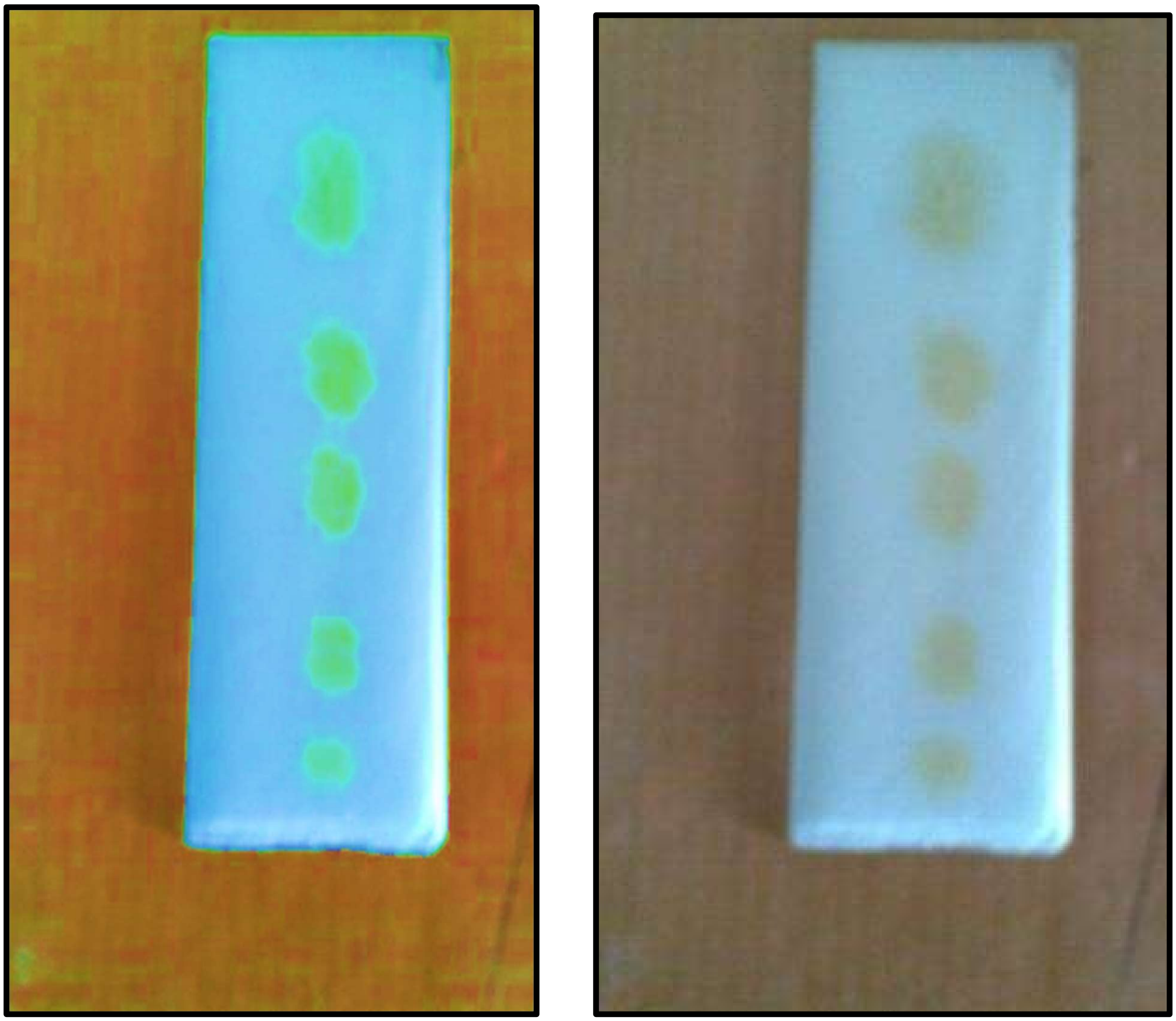


Fig.No.4 Photographic image of spot of

TLC of extract of sesbania grandiflora

Spot No.	R_f Value	Colour of the spot		
		Visually	Under UV (254nm)	Vanillin-H_2SO_4.
1	0.14	Colorless	-	Purple
2	0.26	Colorless	Light blue	Purple blue
3	0.39	Colorless	-	violet
4	0.86	Colorless	-	Purple blue

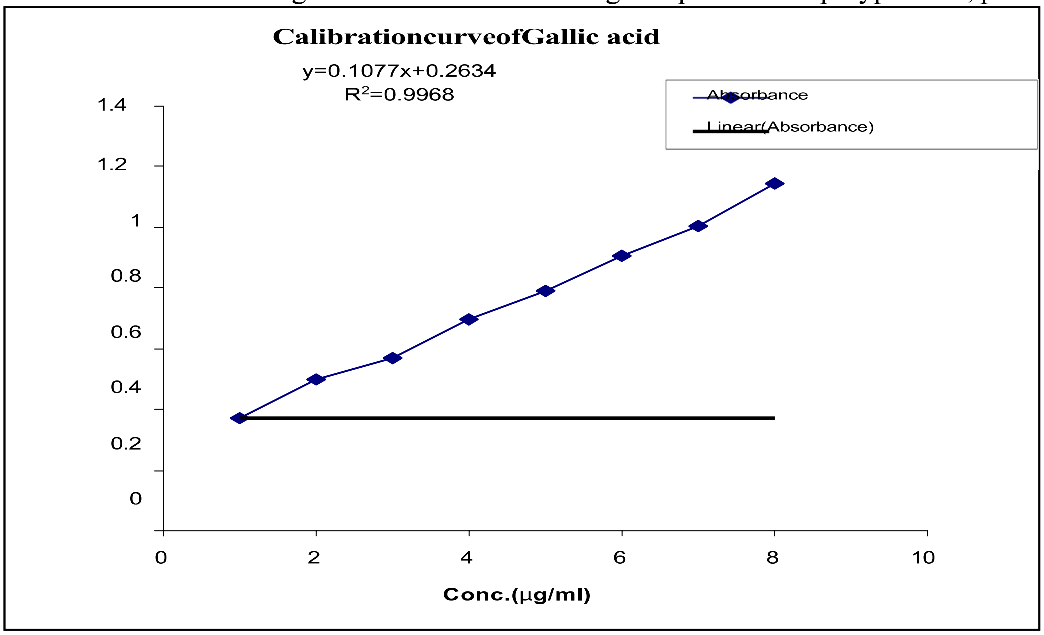
Table No.3: Observation table of TLC of extract of sesbania grandiflora

Chromatographic Conditions
Stationary Phase: Silica gel G

Mobile Phase: Benzene
Length of Run: 10 cm
Chamber Saturation: 30 minutes
Visualizing Agents: UV (254 nm), Vanillin-H_2SO_4

7.4 Total phenolics content

The total phenolic content in the methanolic extracts of Sesbania grandiflora was determined to be 38.04% w/w, respectively, expressed as equivalents to gallic acid.The total phenolic content of the methanol extracts from Sesbania grandiflora, was measured using the Folin-Ciocalteu method. This reagent is sensitive to reducing compounds and polyphenols, producing



a blue-colored complex. The quantitative estimation of phenolics was conducted at a maximum absorbance of 765 nm by monitoring changes in the intensity of the Folin-phenolic compound complex.

FigNo.6: Calibration curve for Gallic acid

7.5 Antioxidant activity by DPPH free radical scavenging assay

The DPPH radical scavenging ability is commonly employed as an indicator to assess the antioxidant potential of medicinal plants. In the DPPH free radical scavenging assay, the petroleum ether extract, chloroform extract, and methanolic extract of Cayratia trifolia, along with ascorbic acid, exhibited DPPH radical scavenging activity, with IC50 values of 144.58 µg/ml, 102.88 µg/ml, 64.51 µg/ml, and 13.86 µg/ml, respectively. Notably, the methanolic extract demonstrated significant scavenging capacity (see Table 4 and Fig. 7).In vitro antioxidant studies on the three extracts of Sesbania grandiflora assessed the DPPH radical scavenging effect at various concentrations (25-100 µg/ml), using ascorbic acid as the standard. The radical scavenging activity was found to increase with higher concentrations. The control and plant extracts exhibited maximum activities of 91.93% for the control, 78.49% for methanol, 50.68% for chloroform, and 34.14% for petroleum ether, with corresponding IC50 values of 13.86 µg/ml, 54.34 µg/ml, 112.08 µg/ml, and 124.75 µg/ml (see Table 4).

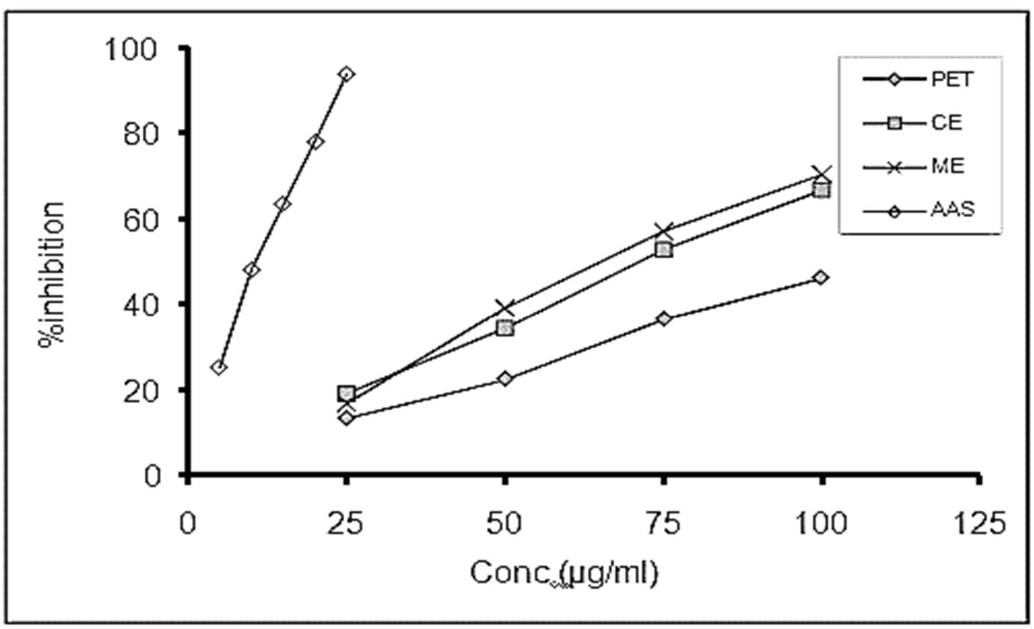
Table No.4: DPPH free radical scavenging activity of extracts of *Sesbania grandiflora*

Test component	Conc. (µg/ml)	%inhibition	IC_{50} (µg/ml)
PetroleumetherExtract (PE)	25	17.07	124.75
	50	25.78	
	75	29.80	
	100	34.03	
ChloroformExtract(CE)	25	13.54	112.08
	50	25.47	
	75	38.13	
	100	50.57	
MethanolExtract(ME)	25	20.52	54.34
	50	34.01	
	75	45.62	
	100	78.38	
Ascorbicacid(ASA)	5	15.42	13.86
	10	34.40	
	15	51.34	
	20	73.76	
	25	91.82	

Fig.No.7: DPPH free radical scavenging activity of extracts of Sesbania grandiflora

7.6 Alloxan induced Diabetes mellitus

Alloxan induces diabetes by selectively destroying the insulin-producing beta cells of the pancreas. In vitro studies have demonstrated that alloxan exhibits specific toxicity toward pancreatic beta cells, resulting in cell necrosis. Administration of alloxan (150 mg/kg, i.p.) caused a 1.5-fold increase in fasting blood glucose levels, which remained elevated for three



weeks. Over this period, daily treatment with the extracts of Cayratia trifolia, Alternanthera sessilis, and Sesbania grandiflora led to a dose-dependent reduction in blood sugar levels.

Specifically, the methanolic extract of Cayratia trifolia and Alternanthera sessilis reduced blood glucose from 290 mg/dl to 233 mg/dl over 21 days (see Table 19 and Fig. 17), while the methanolic extract of Sesbania grandiflora decreased levels from 288 mg/dl to 229 mg/dl during the same time frame (see Table 20 and Fig. 18). The effect of treatment appeared to peak after 15 days and remained stable during the third week. Vehicle control animals maintained stable body weight, whereas diabetic rats experienced a significant reduction in body weight over the 21-day period. Alloxan administration led to this decrease in body weight.

Table No.5: Anti diabetic effect of extracts of Sesbania grandiflora

Sr.No.	Group	Treatment	Fasting blood glucose level(mg/dl)			
			Basalvalue	7thday	14th day	21stday
1	I	Normal Control	90±1.02**	92±1.08**	91±0.92**	93±0.87**
2	II	Diabetic Control (Vehicle)	296±2.12	291±3.04	293±1.62	295±2.64
3	III	Alloxan+glibenclamide (10 mg/kg)	295±2.52**	211±2.74**	158±1.29**	149±1.96**
4	VII	Alloxan+Methanolicextract of *sesbania grandiflora* (100mg/kg)	296±2.14**	265±2.74**	245±2.01**	237±2.47**
5	VIII	Alloxan + Methanolicextractof*sesbania grandiflora* (200mg/kg)	294±1.78**	260±2.75**	239±1.87**	232±1.91**
6	IX	Alloxan + Methanolic extract of *sesbania grandiflora* (400mg/kg)	288±2.45**	254±2.14**	236±1.63**	229±2.41**

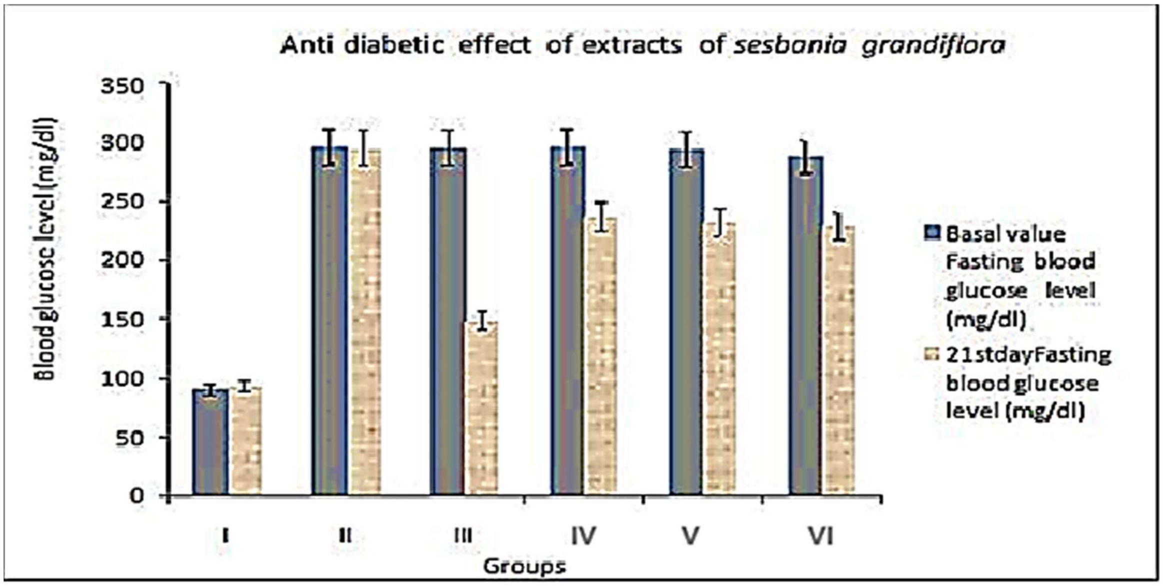


Fig.No. 8 Anti-diabetic effect of extracts of Sesbania grandiflora

Group I Normal Control

Group II Diabetic Control (Vehicle)
Group III Alloxan+ Glibenclamide (10mg/kg)
Group IV Alloxan + Methanolic extract of *sesbania grandiflora* (100mg/kg)
Group V Alloxan+Methanolic extract of *sesbania grandiflora* (200mg/kg)
Group VI Alloxan+Methanolic extract of *sesbania grandiflora* (400mg/kg)

8. Discussion

The phytochemical analysis of these plant extracts uncovered a wide variety of bioactive components, such as tannins, steroids, flavonoids, and other minor components. These chemicals add to the plants' medicinal potential and are typically linked to important biological processes. Total phenolic content was quantitatively assessed in the methanolic extracts, revealing values of 50.72% w/w for Sesbania grandiflora.The antioxidant activity of these compounds is critical in mitigating oxidative stress, which is implicated in various chronic diseases.In vitro assessments of antioxidant capacity, specifically through the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay, demonstrated that the extracts from, along with ascorbic acid, acted as effective scavengers of free radicals. The IC50 values indicated varying degrees of effectiveness, with the methanolic extract displaying superior activity. This antioxidant potential is significant, as reactive oxygen species (ROS) contribute to cellular damage and inflammation, linking oxidative stress to a range of pathological conditions.The potential antidiabetic effects of *Sesbania grandiflora* were evaluated in alloxan-induced diabetic models. Alloxan is known to selectively destroy pancreatic beta cells, resulting in hyperglycemia. The administration of plant extracts resulted in a significant reduction in blood glucose levels over a period of 21 days. Although the antidiabetic efficacy was less potent than that of the standard drug glibenclamide, the findings highlight the potential of these plant extracts in managing diabetes, particularly in populations where traditional remedies are preferred.

9. Conclusion

The extensive pharmacological evaluation of Sesbania grandiflora confirms their multifaceted therapeutic potential, substantiating their historical applications in traditional medicine. The findings advocate for a renewed interest in these plants, particularly in light of contemporary health challenges such as inflammation, infection, and chronic diseases. The integration of traditional knowledge with modern scientific methodologies holds great promise for the development of effective, natural remedies that can contribute significantly to global health solutions. Through continued research and exploration, these plants may serve as a foundation for innovative treatments that address unmet medical needs.

Conflict of Interests The authors have no conflict of interests.

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