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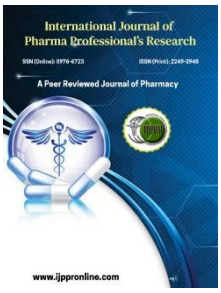

In-vitro Antidiabetic Activity of *Acacia Arabica* Leaves

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ABSTRACT: The aim of the present study was to investigate the phytochemical bioactive compounds of the ethanolic extract of *Acacia Arabica* leaves and its *in-vitro* anti-diabetic activity. The intestinal digestive enzymes alpha-glucosidase and alpha-amylase play a very important role in the digestion of carbohydrates. One antidiabetic therapeutic approach reduces the post-prandial glucose level in blood by the inhibition of alpha-glucosidase and alpha-amylase enzymes. These are important strategies in the management of blood glucose. The results of this assay suggest that the presence of bioactive compounds could be responsible for the versatile medicinal properties of this plant including diabetes, the extract exhibits a dose-dependent increase in inhibitory effect on glucose diffusion (up to 72.03%), and alpha-amylase enzyme (up to 64.84%). The current *in-vitro* study proves that the ethanolic extract of *Acacia Arabica* leaves has anti-diabetic activity.

1. Introduction:

Diabetes is one of the major causes of premature death worldwide. Every ten seconds a person dies from diabetes-related causes mainly from cardiovascular complications. In 2007, diabetes caused 3.5 million deaths globally.[1] Diabetes mellitus is a complex and diverse group of disorders that disturbs the metabolism of carbohydrates, fat and protein. The number of diabetes mellitus cases has been increasing worldwide in recent years. In 2000, the World Health Organization estimated a total of 171 million people with diabetes mellitus from the global population, and this report projected to increase to 366 million by 2030.[2,3]

The genus Acacia is the second largest in the family Leguminosae, with about 1350 species. It is distributed throughout tropical and warm temperate areas of the world, with the largest concentration of species in Australia (957 species), The Americas (185 species), Africa (144 species), and Asia (89 species). *Acacia nilotica* is one of the species that has been effectively utilized in folk medicine for the treatment of tuberculosis, leprosy, smallpox, dysentery, cough, ophthalmia, toothache, skin cancer as astringent,

antispasmodic, and aphrodisiac by rural population.[4]

The potential effects of *Acacia arabica* in relation to diabetes are described further. Some studies have shown that *Acacia arabica* extracts may have hypoglycemic (blood sugar-lowering) effects. These effects are attributed to various phytochemicals found in the plant, such as tannins and flavonoids. These compounds may help regulate blood glucose levels, which is crucial for individuals with diabetes.[5,6]

Acacia arabica contains antioxidants, which can help reduce oxidative stress and inflammation. Diabetes is associated with increased oxidative stress, which can lead to complications. Antioxidants can potentially mitigate these effects. Chronic inflammation is linked to insulin resistance and type 2 diabetes. Some components of *Acacia arabica* may have anti-inflammatory properties, which could be beneficial for people with diabetes.[7]

Acacia arabica gum has been traditionally used to manage gastrointestinal issues. Since diabetes can sometimes affect the digestive system, the plant's gum may provide relief from related symptoms. Obesity is a significant risk factor for type 2 diabetes. Some compounds in *Acacia arabica* may contribute to weight management by promoting a feeling of fullness or reducing appetite, although this effect is not well-established.[8,3]

2. Materials and method:

2.1 Collection of raw material: The plant leaves were procured from Hapur District in Uttar Pradesh. The plant was authentified as *Acacia arabica* by Dr. Sunita Garg (Chief scientist), National Institute of Science Communication and Information Resources (NISCAIR). Pusa campus, New Delhi. A voucher specimen of the plant is preserved in the herbarium. Ref.No. (NISCAIR/RHMD/Consult/2020/3670- 71).

2.2 Preparation of extract: Leaves of *Acacia arabica* were dried under shade and they uniformly grounded using a mechanical grinder to yield coarse powder. Powdered material was extracted at 50-55° C for a time overnight to extract all ethanol-soluble constituents. The powder (100 gm) was extracted with ethanol (300 ml) for 72 hrs. The filtrate obtained was concentrated by removing the solvent by distillation under reduced pressure. The temperature range for the extraction is 1550C ± 50C. The time required for the complete extraction of *Acacia arabica* depends on the temperature used for the extraction. The extract was cooled and kept in desiccators overnight. The ethanol extract was weighed and used for the study. The % yield of *Acacia arabica* was found to be 12% w/w on a dry basis.

2.3 Chemicals and instrument required: Ethanol, and other chemicals used for extraction purposes and phytochemical tests were of laboratory grade (LR) reagents, sonicator, soxhlet distillation unit

3. Experimental Methodologies: 3.1 Preliminary phytochemical screening: The leaves of *Acacia arabica* were subjected to preliminary phytochemical screening for the detection of various plant constituents. A standard screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, flavonoids, carbohydrates, phenolic compounds, saponins, steroids, tannins, etc. by using standard procedures.[4]

3.2 Phytochemical analysis:

3.2.1 Test for alkaloids:

3.2.1.1 Wagner's test: About 10 mg of leaf extract was taken and a few drops of Wagner's reagent was added and the formation of a reddish-brown precipitate indicates the presence of alkaloids.

3.2.2 Test for flavonoids:

3.2.2.1 Shinoda test: 10 mg of leaf extract was added to a pinch of magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. The formation of a pink color indicates the presence of flavonoids.

3.2.2.2 Lead acetate test: 10 mg of leaf extract was taken and a few drops of 10% lead acetate solution were added. The appearance of a yellow color precipitate indicates the presence of flavonoids.

3.2.3 Test for phenols and tannins:

3.2.3.1 Lead acetate test: 10 mg of leaf extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of a precipitate indicates the presence of tannins and phenolic compounds. Ferric chloride test-5 mg of leaf extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish-black color indicates the presence of tannins.

3.2.3.2 Sodium hydroxide test: 5 mg of extract was dissolved in 0.5 ml of 20% sulphuric acid solution. Followed by the addition of a few drops of aqueous sodium hydroxide solution, it turns blue which indicates the presence of phenols.

3.2.4 Test for carbohydrates:

3.2.4.1 Fehling's test: 5 ml of Fehling's solution was added to 0.5 mg of leaf extract and boiled in a water bath. The formation of a yellow or red precipitate indicates the presence of reducing power.

3.2.4.2 Benedict's test: 5 ml of Benedict's solution was added to 0.5 mg of extract and boiled in a water bath. The appearance of red or yellow or green precipitate indicates the presence of reducing sugars.

3.2.5 Test for saponins:

3.2.5.1 Honeycomb test: 0.5 mg of leaf extract was taken in a test tube and a few drops of 5% sodium bicarbonate solution was added. The mixture was shaken vigorously and kept for 3 min. The formation of honeycomb-like froth shows the presence of saponins.

3.2.5.2 Foam test: 0.5 mg of extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for 15 min. The formation of foam to a length of 1 cm indicated the presence of saponins.

3.2.6 Test for glycosides:

Glycoside test: 0.5 mg of leaf extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. The formation of a yellow color indicates the presence of glycosides.

Table 1. Phytochemical analysis of *Acacia arabica* extract

Phytochemical test	**Extract**			
	Ethanol	**Acetone**	**Chloroform**	**Name of test**
Alkaloids	+	-	+	**Wagner's test**
Flavonoids	-	+	+	**Shinoda test**
Phenol and tannins	-	+	-	**Lead acetate test**
Carbohydrates	+	-	-	**Fehling's test**
Saponins	+	-	+	**Honey comb test**
Glycosides	+	+	-	**Glycoside test**

(+) Present (-) Absent

3.3 Methods for *in-vitro* Antidiabetic Study:

3.3.1 Alpha-amylase inhibition: In this assay, 390 µl of 0.02 M phosphate buffer (pH 7), positive control (acarbose), different concentrations of test samples and 10 µl of α-amylase enzyme were mixed and incubated at 37°C for 10 min. Added 10 µl of starch to this mixture and re-incubated 37°C for 1 h. After incubation, 0.1 ml of 1% iodine solution and 5 ml of distilled water were added and optical density was measured at 565 nm. Inhibition of enzyme activity was calculated as follows[9]:

Percentage inhibition = (A−C) × 100/ (B−C)

Where,

A=Absorbance of the sample,

B=Absorbance of blank
C= Absorbance of sample

3.3.2 Glucose diffusion inhibition: 1 ml of the extract was placed in a dialysis membrane along with a glucose solution (0.22 mM in 0.15 M sodium chloride). It was then tied at both ends using thread and it was immersed in a beaker containing 40 ml of 0.15 M sodium chloride and 10 ml of distilled water. 1 ml of 0.15 M sodium chloride containing 22 mM glucose and 1 ml of distilled water, the beakers were then placed on an orbital shaker and kept at room temperature. The movement of glucose into the external solution was monitored by measuring the glucose concentration in the external.

4. Result and Discussion:

4.1 *In-vitro* antidiabetic activity

4.1.1 Alpha-amylase inhibition: The *in-vitro* α-amylase inhibitory studies demonstrated that the methanolic extract of *Acacia arabica* at concentrations of 25, 50, 75 and 100 µg / ml produced a significant percentage inhibition. The highest concentration of 100 µg/ml produced a maximum inhibition of 64.84% as compared to standard acarbose which showed a significantly higher inhibition of 66.74% at the concentration of 100 µg / ml.

Table 2: *In-vitro* anti-diabetic effect of leaves of *Acacia arabica*

Concentration µg/ml	AAE (ethanol) % inhibition	Standard Acarbose % inhibition
25 µg/ml dilution	50.66±1.85*	52.33±1.45
50 µg/ml dilution	55.67±2.72**	57.33±2.90
75 µg/ml dilution	60.56 ± 3.4	62.56 ± 3.4
100 µg/ml dilution	64.84 ± 3.3**	66.74 ±2.89

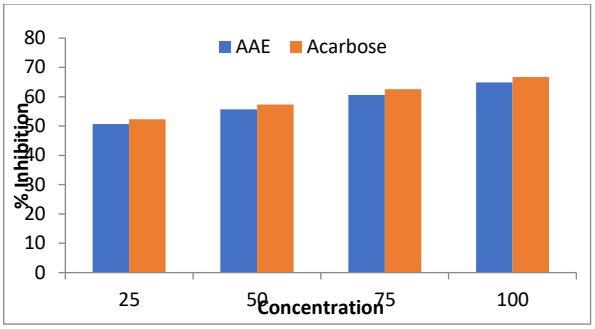

Figure 1: Determination of percentage inhibition of alpha-amylase.

4.1.2 Glucose diffusion inhibition: The level of inhibition of glucose movement by the plant extract at various time intervals of 30, 60, 90 and 120 min was evaluated and compared with the standard acarbose at the same concentration. From the study, it was observed that at 120 min, the extract produced a significant inhibition of 70.03% at 400 µg/ml when compared with standard acarbose which had a higher inhibition of 77.26% at 400 µg /ml.

Table 3: *In-vitro* anti-diabetic effect of leaves of *Acacia arabica*

Time intervals	AAE (ethanol) % inhibition	Standard Acarbose % inhibition
30 Minute	60.66±1.85*	63.33±1.45
60 Minute	63.67±2.72**	66.33±2.90
90 Minute	65.56 ± 3.4	70.56 ± 3.4
120 Minute	70.03 ± 3.3**	77.26 ±.89

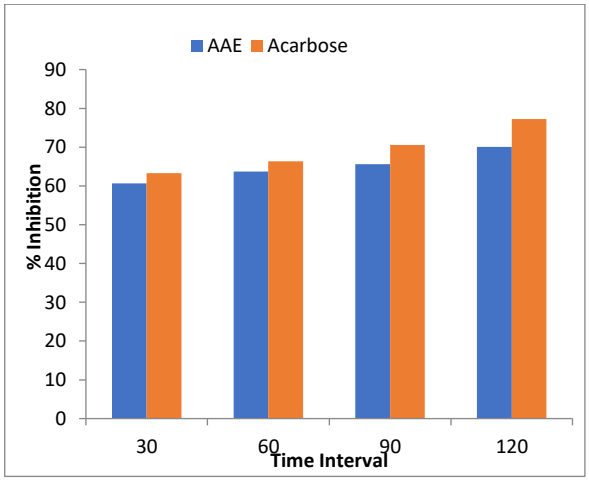

Figure 2: Determination of percentage inhibition of Glucose diffusion.

5. Conclusion: *Acacia arabica* is a well-known plant among the users of traditional practitioners as well as the present researchers of medicines. We upon screening the leaves parts of the plant for the antioxidant activity conceded to the following conclusion. Diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defenses. The % yield of *Acacia arabica* was found to be 12% w/w on a dry basis. Preliminary phytochemical screening indicated the presence of active compounds such as alkaloids and flavonoids. it has been reported that chemical compounds such as alkaloids and flavonoids present in *Acacia arabica* play a major role in preventing diabetes. In antioxidant activity, DPPH scavenging activity Methods of 100 µg/ml extracts show better % inhibition as compared to control and 50 µg/m extracts. In DPPH Scavenging activity % inhibition of 100 µg/ml ethanol extracts was 67.30%. The ethanol extract showed strong antioxidant activity with the DPPH radical scavenging experiment. Experimental results showed that the extract is capable of alleviating the augmented oxidative state associated with diabetes. It was concluded that administration of *Acacia arabica* leaf extract was effective in decreasing the blood glucose level.

6. References

1. Das AK, Rai M, A world without diabetes and its complications: A preventive program, Type. 2008; 2: 1.
2. Wild S, Roglic G, Green A, Sicree R, King H, Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27(5): 1047-53.
3. Sharma AK, Kumar A, Yadav SK, Rahal A, Studies on antimicrobial and immunomodulatory effects of hot aqueous extract of Acacia nilotica L. leaves against common veterinary pathogens, Veterinary Medicine International. 2014; 2014.
4. Geetha TS, Geetha N, Phytochemical screening, quantitative analysis of primary and secondary metabolites of Cymbopogan citratus (DC) Stapf. leaves from Kodaikanal hills, Tamilnadu, International Journal of Pharmtech Research. 2014; 6(2): 521-9.
5. Gotama TL, Husni A, Antidiabetic activity of Sargassum hystrix extracts in streptozotocin-induced diabetic rats, Preventive Nutrition and Food Science. 2018; 23(3): 189.
6. Deshpande SN, Preliminary Phytochemical Analysis and In Vitro Investigation of Antibacterial Activity Of Acacia Nilotica Against Clinical Isolates, Journal of Pharmacognosy and Phytochemistry. 2013; 1(5): 23-7.
7. Kifle ZD, Yesuf JS, Atnafie SA, Evaluation of in vitro and in vivo anti-diabetic, anti-hyperlipidemic and anti-oxidant activity of flower crude extract and solvent fractions of hagenia abyssinica (rosaceae), Journal of Experimental Pharmacology. 2020: 151-67.
8. Ouassou H, Bouhrim M, Bencheikh N, Addi M, Hano C, Mekhfi H, Ziyyat A, Legssyer A, Aziz M, Bnouham M, In vitro antioxidant properties, glucose-diffusion effects, α-amylase inhibitory activity, and antidiabetogenic effects of C. Europaea extracts in experimental animals, Antioxidants. 2021; 10(11): 1747.
9. Narkhede MB, Ajimire PV, Wagh AE, Mohan M, Shivashanmugam AT, In vitro antidiabetic activity of Caesalpina digyna (R.) methanol root extract, Asian Journal of Plant Science and Research. 2011; 1(2): 101-6.