

ANTI DIABETIC POTENTIAL OF PLANT ABELMOSCHUS ESCULENTS AND IN VITRO STUDIES OF ANTI DIABETIC ACTIVITY ON PLANT ABELMOSCHUS ESCULENTS

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ABSTRACT

Diabetes mellitus is a heterogeneous group of disorders characterized by increasing blood glucose level caused by lack of insulin hormone. One of the plants that were often used as traditional medicine for diabetes in Indonesia was okra (*Abelmoschus esculentus* L.) leaves. The aim of this research was to evaluate the antidiabetes activity of okra fruit extract. The study was divided into several steps: a preliminary study of anthyperglycemia of okra leaves after glucose, sucrose, and amylum administration; antidiabetic activity in insulin deficiency animal model developed by aloxan administration and insulin resistance animal model developed by lipid emulsion administration, and in vitro study of α -glucosidase and α -amylase enzyme inhibition activity. The result showed that the extract group had antihyperglycemia activity after glucose, sucrose, and amylum administration. In insulin deficiency animal model, glibenclamide and extract could decrease blood glucose level. In insulin resistance

animal, metformin and extract could increase constantly of insulin tolerance test. From the α -glucosidase inhibition activity, it was known the acarbose's IC₅₀ was 44.374 μ g/mL, while okra leaves extract was 1533.742 μ g/mL. And the IC₅₀ value of acarbose to α -amylase enzyme was 46.821 μ g / mL, while okra leaves extract was 1228.469 μ g / mL.

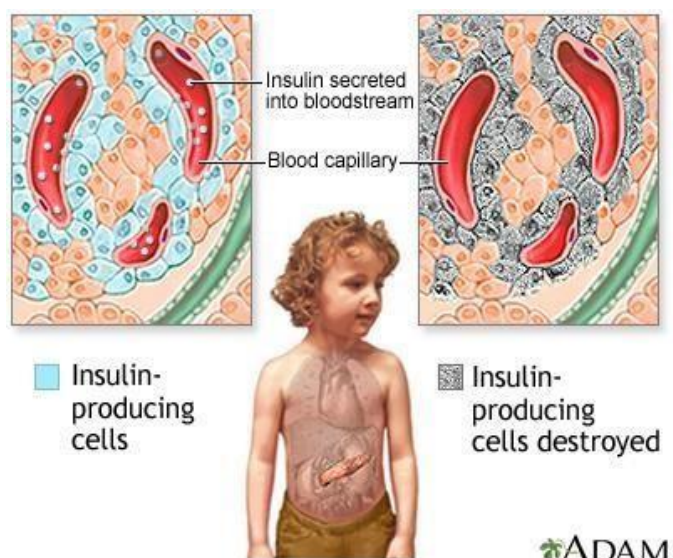
KEYWORDS: *Abelmoschus esculentus* L., diabetes mellitus, insulin deficiency, insulin resistance, okra leaves.

INTRODUCTION

Traditional Medicines derived from medicinal plants are used by about 60% of the world's population. This review focuses on Indian Herbal drugs and plants used in the treatment of diabetes, especially in India. Diabetes is an important human ailment afflicting many from various walks of life in different countries. In India it is proving to be a major health problem, especially in the urban areas. One of the etiologic factors implicated in the development of diabetes and its complications is the damage induced by free radicals and hence an antidiabetic compound with antioxidant properties would be more beneficial. Therefore, information on antioxidant effects of these medicinal plants is also included. Diabetes is the disease or disorder of pancreas by which pancreas stop the secretion of insulin in the body. Insulin allows the glucose enter in to the cells which provide energy to every cellof the body without insulin, glucose cannot enter in tothe cell. Those part which helps to secreteinsulin, the part which are defective which is known as diabetes type 1. In the type of diabetesmellitus, the overall diabetes cases 10%. The type-1 diabetes which is most occurring in children. About 90% of incidence of type-2 diabetes mellitus. It is mainly occurring after the age 40s.

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism. Firstly, the presence of sugar in the urine of Diabetics was demonstrated by Dobson in 1755. In 1989 von Mering and Minkowski that pancreatectomized dose become diabetic in addition to developing digestive disturbances. It is a group of metabolic disorder characterized by high blood sugar level.^[2]

It is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period This high blood sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolarcoma. Serious long-term complications include heart disease, stroke, kidney failure, foot ulcersand damage tothe eyes. Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produce.^[3]



Diabetes mellitus types

1. **Type 1 Diabetes:** It is autoimmune disease, in this type of diabetes generally occurs due to the destroy of beta cell in the pancreas where insulin is formed.
2. **Type 2 Diabetes:** It occurs when our body resistance or secreted the low amount of insulin thus increases the sugar level in your blood, because insulin manage the blood sugar level. In this condition of blood glucose level may rises up to 6-20 time of the normal range and altered the state develop or person loss of function.

DIABETES MELLITUS

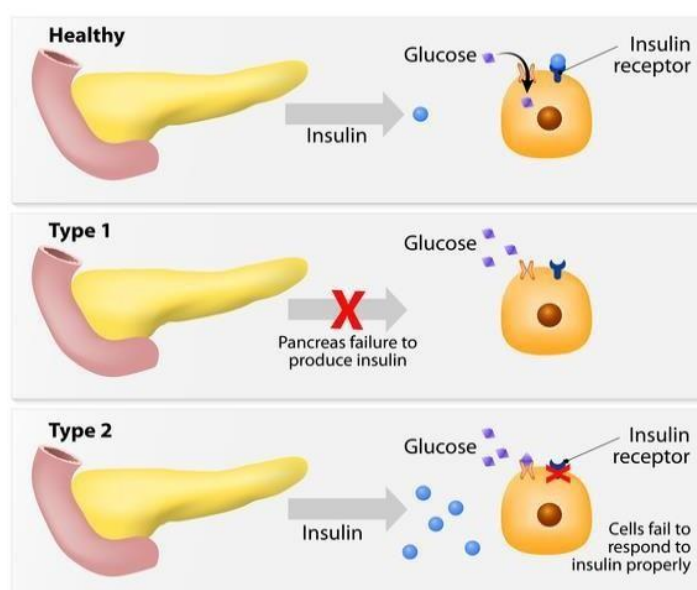


Figure 2: Healthy pancreas and pancreas in type 1 and type 2 diabetes mellitus.

- 2- **Pre diabetes:** It occurs when your blood sugar is higher than normal range, but it is not high to enough for a diagnosis of Type 2 diabetes.

3- Gestational diabetes: It is high blood sugar during the pregnancy. It causes due to insulin-blocking hormone producing by the placenta. After baby is born, gestational diabetes usually goes away. Gestational diabetes makes you more likely to develop Type2 diabetes.

Note: According to current definition of D M, two fasting glucose measurement above 126 is considered diagnostic for D M. Per the WHO people with fasting glucose levels from 110 to 125mg/dl are considered to have impaired fasting glucose HbA1c test is much better than the FGP test for determination risk of cardiovascular disease and death from any cause.

Estimation of Diabetes

The World Health Organization estimated that about 30 million people suffered from diabetes in 1985 and the number increased to more than 171 million in 2000. It is estimated that the number will increase to over 366 million by 2030 and that large increases will occur in developing countries, especially in people aged between 45 and 64 years.^[5]

PLANT PROFILE



1. GENERAL DESCRIPTION

Okra *Abelmoschus esculentus* L. (Moench), is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. This crop is suitable for cultivation as a garden crop as well as on large commercial farms. It is grown commercially in India, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Burma, Japan, Malaysia, Brazil, Ghana, Ethiopia, Cyprus and the Southern United States. India ranks first in the world with 3.5 million tonnes (70% of the total world production) of okra produced from over 0.35 million hectare land (FAOSTAT, 2008).

Name	Okra
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Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Malvales
Family	Malvaceae
Genus	<i>Abelmoschus</i>
Species	<i>esculentus</i>

2. Geographical Origin and Distribution

Cultivated and wild species clearly show overlapping in Southeast Asia, which is considered as the centre of diversity. The spread of the other species is the result of their introduction to America and Africa. There are two hypotheses concerning the geographical origin of *A. esculentus*. Some authors argue that one putative ancestor (*A. tuberculatus*) is native to Uttar Pradesh in northern India, suggesting that the species originated from this geographic area. Others, on the basis of ancient cultivation in East Africa and the presence of the other putative ancestor (*A. ficulneus*), suggest that the area of domestication is north Egypt or Ethiopia, but no definitive proof is available today. For *A. caillei*, only found in West Africa, it is difficult to suggest an origin outside. Its origin by hybridization with *A. manihot* is difficult to accept even if its presence, mentioned in the Flora of West Africa (Hutchinson and Dalziel, 1958) was not recently confirmed in this area and herbarium samples are lacking.

Eight *Abelmoschus* species occur in India. Out of these, *A. esculentus* is the only known cultivated species. *A. moschatus* occurs as wild species and is also cultivated for its aromatic seeds, while the rest six are truly wild types. The wild species occupy diverse habitats. The species *A. ficulneus* and *A. tuberculatus* is spread over the semi-arid areas in north and northwestern India; *A. crinitus* and *A. manihot* (*tetraphyllus* and *pungens* types) in tarai range and lower Himalayas; *A. manihot* (*tetraphyllus* types), *A. angulosus*, and *A. moschatus* in Western and Eastern Ghats; and *A. crinitus* and *A. manihot* (mostly *pungens* types) in the northeastern region, depicting their broad range of distribution in different phytogeographical regions of the country. Intra as well as interspecific variations do exist in different phyto-geographic areas. Existence of different *Abelmoschus* species in different areas of India observed in a recent survey is presented in Table.

Table: Distribution of Wild *Abelmoschus* Species in different Phytogeographical Regions of India.

S.No.	Species	Distribution
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1.	<i>A. angulosus</i>	Tamil Nadu, Kerala
2.	<i>A. cancellatus</i>	Uttaranchal, Himachal Pradesh, Uttar Pradesh, Orissa
3.	<i>A. crinitus</i>	Uttaranchal, Madhya Pradesh, Orissa
4.	<i>A. ficulneus</i>	Jammu & Kashmir, Rajasthan, Madhya Pradesh, Chhattisgarh, Maharashtra, Tamil Nadu, Andhra Pradesh, Uttar Pradesh
5.	<i>A. manihot</i> ssp. <i>tetraphyllus</i> var. <i>tetraphyllus</i>	Uttar Pradesh, Rajasthan, Madhya Pradesh, Maharashtra, Orissa, Chhattisgarh
6.	<i>A. manihot</i> ssp. <i>tetraphyllus</i> var. <i>pungens</i>	Uttaranchal, Himachal Pradesh, Jammu & Kashmir, Assam, Andaman & Nicobar Islands
7.	<i>A. moschatus</i> ssp. <i>moschatus</i>	Uttaranchal, Orissa, Kerala, Karnataka, Andaman & Nicobar Islands
8.	<i>A. moschatus</i> ssp. <i>tuberosus</i>	Kerala and parts of Western Ghats in Tamil Nadu
9.	<i>A. tuberculatus</i>	Uttar Pradesh, Rajasthan, Madhya Pradesh, Maharashtra

Source: Bisht and Bhat, 2006

3. Growth and Development

Okra is mainly propagated by seeds and has duration of 90-100 days. It is generally an annual plant. Its stem is robust, erect, variable in branching and varying from 0.5 to 4.0 metres in height. Leaves are alternate and usually palmately five lobed, whereas the flower is axillary and solitary. The botanical features of various plant parts are detailed in Annexure –I.

Okra plants are characterized by indeterminate growth. Flowering is continuous but highly dependent upon biotic and abiotic stress. The plant usually bears its first flower one to two months after sowing. The fruit is a capsule and grows quickly after flowering. The greatest increase in fruit length, height and diameter occurs during 4th to 6th day after pollination. It is at this stage that fruit is most often plucked for consumption. The okra pods are harvested when immature and high in mucilage, but before becoming highly fibrous. Generally the fibre production in the fruit starts from 6th day onwards of fruit formation and a sudden increase in fibre content from 9th day is observed (Nath, 1976). Okra plants continue to flower and to fruit for an indefinite time, depending upon the variety, the season and soil moisture and fertility. Infact the regular harvesting stimulates continued fruiting, so much that it may be necessary to harvest every day in climates where growth is especially vigorous.

BOTANICAL FEATURES OF OKRA

Okra is an upright annual, herbaceous 3 to 8 feet tall plant with a hibiscus-like flower. It is a tropical direct sown vegetable with a duration of 90-100 days. The botanical features are as

indicated below:

Root

Okra plant has a deep taproot system.

Stem

Its stem is semi woody and sometimes pigmented with a green or reddish tinges color. It is erect, variable in branching, with many short branches that are attached to thick semi woody stem. The stem attains height from 3 feet in dwarf varieties to 7 or 8 feet in others.

Leaves

The woody stem bear leaves that are lobed and are generally hairy, some reaching up to 12 inches in length. Leaves are cordate (heart-shaped), simple, usually palmately 3-7 lobed and veined. Leaves are subtended by a pair of narrow stipules. The okra leaf is dark green in color and resembles a maple leaf.

Flowers

The flowers are borne vertically only on the orthotropic axis every two or three days. The flower is axillary and solitary, borne on a peduncle 2.0 – 2.5 cm long. The flowers are large around 2 inches in diameter, with five white to yellow petals with a red or purple spot at the base of each petal. Flower lasts only for a day. Each blossom develops a small green pod. The flowers are almost always bisexual and actinomorphic. The perianth consists of 5 valvate, distinct or basally connate sepals and 5 distinct petals that are usually basally adnate to the androecium.

The androecium consists numerous monadelphous stamens with apically divergent filaments bearing 1-celled anthers.



FRUIT

The fruit is an elongated, conical or cylindrical capsule, comprising for the most part, five cavities containing ovules (Figure 5). The fruit is actually long pod and generally ribbed,

developing in the leaf axil and spineless in cultivated kinds. The fruit is normally yellowish green to green, but is sometimes purple or whitish green. The pods are the edible portion, which are harvested while still tender and immature. They grow rapidly into long (10-30 cm) and narrow (1-4 cm) pod with a tip that is either pointed like a beak or blunt.



EXPERIMENTAL

Identification and Authentication of Plant Material

Okra (*Abelmoschus esculentus* L.) leaves was obtained from Research Station for Spices and Medicinal Plants, Bogor, West Java, Indonesia. The plant identification and authentication were performed by Center for Plant Conservation Botanic Gardens, Bogor, West Java, Indonesia.

Preparation of *Abelmoschus esculentus* L. Extract

Okra fruit was cleaned and cut into pieces, then mashed using a blender with enough water. The mixture was filtered using a filter paper and the filtrate was concentrated using freeze drier.

Animals

Male Swiss–Webster mice 2-3 months old weighing 20-30 g were kept at standard laboratory conditions at 24-26°C, humidity 70-75%, and 12 hours light/dark cycle. Animals were fed with standard chow and water *ad libitum*. The methods in this study were performed in accordance with ethics and guide for animals care and used. Ethical approval was published by the Ethics Committee Padjajaran University with registration number 0818020269.

GENERAL PROCEDURE

Antihyperglycemic Evaluation as a Preliminary Study

Before the commencement of the experiment and after first stage induction using high carbohydrate diet, an oral glucose tolerance test (OGTT) using glucose 3 g/kg bw was

performed after a 12-hour fasting period. Blood glucose concentration from the tail vein was measured using the Easy Touch® blood glucose meter at 0 and 30, 60, 90 and 120 min after glucose administration.^[18] The modification of this method was also performed by replacing the glucose with sucrose or starch.

***In-vivo* Antidiabetic Evaluation of Okra Fruit Extract in Insulin Deficiency Animal Model**

There were two stages in this experiment, the first one was the induction phase and the second was the treatment phase. In the first phase, mice were induced with alloxan 50 mg / kg bw intravenously.^[19] Mice with hyperglycemia (blood glucose > 200 mg/dL) would be used in the second phase of evaluation. Mice were randomly divided into 7 groups: negative control group, the positive control group, standard drug group (glipizide 0.65 mg/kg bw), and extract groups at a dose of 25 mg/kg bw, 50 mg/kg bw, 100 mg/kg, and 200 mg/kg bw. Administration of extracts and glipizide as a standard drug were performed for 14 days. Parameters measured were blood glucose levels on days 3, 7, 11 and 14 during treatment. Mice blood was taken from the vein part of the tail and measured using EasyTouch® glucometer. The data were then analyzed statistically using ANOVA method.

Table 1: Blood Glucose Level after Glucose Administration.

Group	Blood glucose level at minutes- (mg/dl)					
	0	30	60	90	120	150
Negative control	95±3	103±2*	90±5*	76±1*	71±1*	80±11*
Positive control	91±3	207±58	165±6	147±13	129±10	118±23
Glipizide 0.65 mg/kg bw	89±13	98±1*	87±7*	66±9*	65±10*	65±18*
OFE 25 mg/kg bw	90±5	127±1*	109±16*	106±25*#	85±5*	79±10*
OFE 50 mg/kg bw	80±9	98±40*	97±33*	89±23*	75±19*	74±19*
OFE 100 mg/kg bw	80±9	149±43	102±37*	86±24*	88±41*	76±36*
OFE 200 mg/kg bw	84±4	153±37	105±17*	93±15*	66±20*	61±14*

Note: Data are presented as mean ± SD, * means significantly different compared to the positive control group, OFE means okra fruit extract, p < 0.05, n = 3 mice/group.

RESULTS AND DISCUSSION

From the results of this experiment, the following data were obtained: profiles of blood glucose levels in normal animals after administration of glucose, sucrose, and starch; profile of blood glucose levels in animal models of insulin deficiency for 14 days of treatment; profile of blood glucose levels in animal models of insulin resistance for 14 days of treatment; and IC₅₀ values of the inhibition of alpha glucosidase and alpha amylase enzymes.

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