

## ***In-vitro anti diabetic studies and phytochemical evaluation of Heracleum candolleanum***

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### **ABSTRACT**

*The present scientific investigation deals with the identification of chemical constituents from the seed of *Heracleum candolleanum* using specific chemical tests. In the in-vitro anti-diabetic studies were also performed so as to ensure the biological potency of the plant. From the study we revealed that the seed of the plant contains various classes of secondary metabolites and also the plants possess a moderate anti-diabetic activity in terms of alpha amylase inhibition.*

**Keywords:** *Heracleum candolleanum*, anti-diabetic, alpha amylase, EC 50

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### **INTRODUCTION**

Food is any substance consumed to provide nutritional support for the body. Forests are repository of variety of foods tribal living as a part of nature exploited nature to meet their foods are uncommon to us and nutritionally superior and can be selectively used for bringing about better varieties. Plants have provided man with his needs of interims of shelter. Clothing food flavors and fragrance [1]. Natural anti oxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods [5]. Anti oxidant activity plant is due to the presence of flavones, iso flavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins [2].etc many of them anti microbial activity also.

The antis microbial activity was checked by disc diffusion method [3].millets are high energy, nutrition food comparable to other cereals and some of them are even better with regard to protein and mineral. Bamboo seeds are nutrient rich, the overall nutritive quality is slightly greater than the rice and wheat. Bamboo is the most marvelous plant in nature and can be used as roof [4].

There are approximately 60 species of *Heracleum candolleanum*. It is a temperate genus and grows in the warm temperature regions. About 23 species occur in India. *Heracleum candolleanum* (Wight et Arn), Gamble, an endemic species of western Ghats, is a large potential herb with tuberous root commonly found in the hills and mountains of peninsular India at higher altitudes. The plant is used in folk and tribal medicine for various purposes. The Kani tribes administer decoction of the whole plant internally for nervous disorder inflammatory conditions [7].

### **ANTI -DIABETIC MEDICATION:**

Drugs used in diabetes treat diabetes mellitus by lowering glucose level in the blood. With the exceptions of insulin exenatide, liraglutide, and pramlintide all are administered orally and are thus called oral glycemic agents or oral

anti hyperglycemic agents. These are different classes of anti diabetic drugs and this selection depends on nature of the diabetes, age, and situation of the person as well as other factors.

#### DIABETES MELLITUS TYPE 1:

It is a disease caused by the lack of insulin. Insulin must be used in type 1 which must be injected.

#### DIABETES MELLITUS TYPE 2:

It is a disease of insulin resistance by cell. Type 2 is the most common type of diabetes. Treatments include

- agents that increase the amount of insulin secreted by pancreas.
- agents that increase the sensitivity of target organs to insulin.
- agents that decrease the rate at which the glucose is absorbed from the GIT . Several groups of drugs mostly given by mouth are effective in type 2.

**Comparison of anti diabetic medication[6][7]:** sulfonylurea (glyburid, glimipride, glipizide) stimulating insulin release by pancreatic beta cells by inhibiting the K ATP channel. Metformin: acts on liver to cause decrease in insulin resistance.

Alpha glucosidase inhibitor (acarbose, miglitol, voglibose): reduces glucose absorbance by acting on small intestine to cause decrease in production of enzymes needed to digest carbohydrates. Thiazolidinedione (pioglitazone, rosiglitazone): Reduce insulin resistance by activating PPAR-GAMA in fat and muscle. Most anti-diabetic agents are contraindicated in pregnancy, in which insulin is preferred[8].

Biguanides: It reduce hepatic glucose output and increase uptake of glucose by the periphery including skeletal muscle. Although it must be used with caution in patient with impaired liver or kidney function. Typical reduction in glycated hemoglobin values for metformin is 1.5 – 2%. Phenformin (DBI) was used from 1960s through 1980s, but was withdrawn due to lactic acidosis risk [9].

### MATERIALS AND METHODS



Heracleum candolleanum (Inset- Seed)

#### HERACLEUM CANDOLLEANUM

Heracleum is a genus about 60 species (depending on taxonomic interpretation) of biennial and perennial herbs in the carrot, family Apiaceae. They are found throughout the temperate northern hemisphere and in high mountains as far as south Ethiopia. Common names for the genus or its species include hog weed and cowparnep

Synonym: *Heracleum candolleanum*.

Scientific name- *Heracleum candolleanum* gamble

Common names in Malayalam: kattumally, njara, kattugeerakam

These plant in kingdom plantae, phylum: magnoliophyta, Class-magnoliatae, angiosperm, unranked eudicots and asterids belonging to the family apiaceae, order apiales, genus: heracleum.

Flowering class: dicot ; habit: herb[9].

#### **HABIT AND DISTRIBUTIONS**

General habit: Shola forests and grasslands [10]. Global distribution in southern west Ghats and in India it is found in Kerala state districts: palakkad, idukki,thiruvananthapuram, Thrissur, wayanad.

Karnataka state: Mysore, chikmagalur.

Tamil nadu: coimbatore, kanniyakumari, Nilgri[10].

Shurbs, stem ridged. Leaves 35-45×20-25cm, 1 or 2 pinnate; leaflets 7 to 9 or more, lobed, apex acute, serrate, scabrous, coriaceous. umbels compound, terminal, corymbose; secondary peduncles to 6cm long; rays 13, each 3-5cm long, spreading; pedicels 6-10mm long, many- together, spreading. Flowers 5- merous, 2mm across, calyx-5 toothed; petels-3, ovate, acuminate, with a strong midrib, glabrous; stamens 5 free, anthers ovate; ovary compressed. Fruit 7-10×4-7mm, biconvex, dorsally compressed winged glabrous [13].

**MAJOR SPECIES:** *Heracleum mantegazzianum*, *heracleum sphondylium*, *heracleum lantum*, *heracleum aconitifolium*, *heracleum albovii*, *heracleum algerense*, *heracleum armoricum*, *heracleum asperum*, *heracleum carneiflorum*....etc

#### **COLLECTION OF THE MATERIAL**

The samples were collected from natural resources from South India and authenticated from the Taxonomy Department of Uwin Life Sciences, Malappuram, and Kerala.

#### **PREPARATION OF PLANT EXTRACTS AND PHYTOCHEMICAL SCREENING**

The dried seed of *Heracleum candolleanum* subjected for air dried and make up to coarse powder form. Bark powder was extracted successively with methanol using Reflux apparatus. All the extracts were filtered using cotton plug followed by filter paper. The extract were concentrated and dried. The Sextract was stored in air tight container. The bark extract of *Heracleum candolleanum* were analyses for the presence of phenols and anthraquinones.

#### **PRELIMINARY PHYTOCHEMICAL SCREENING OF THE HERACLEUM CANDOLLEANUM**

- The seeds of *Heracleum candolleanum* is taken 10g in 50ml methanol and subjected to extraction. The filtrate was subjected to Moloch's test. Formation of reddish brown ring indicated the presence of carbohydrates.

Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution [brown color indicated the presence of carbohydrate.]

- Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapors. Blue coloration of the spot indicated the presence of phenols.

Test for flavonoids :Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added .A pink or red coloration of the solution indicated the presence of flavonoids in the drugs.

- .Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added. Flocculent white precipitate indicated the presence of flavonoids.

Test for tannins

- Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the drug.

Test for steroid/terpenoid

- Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to 3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids.

Test for alkaloids

- Draggendorf's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Draggendorf's reagent. Orange coloration of the spot indicated the presence of alkaloids.
- Hager's test: The extract was treated with few ml of Hager's reagent. Yellow precipitation indicated the presence of alkaloids.
- Wagner's test: The extract was treated with few ml of Wagner's reagent. The reddish brown precipitation indicated the presence of alkaloids.

#### Tests for Glycosides

- Legal's test: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution. Pink to red color solution indicates the presence of glycosides.

#### Test for Saponins

- Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes. A 1cm layer of foam formation indicates the presence of Saponins

#### Test for Anthraquinones

- Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. Pink or red coloration of aqueous layer indicated the presence of Anthraquinones.

#### Test for Amino acids

- Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent. Blue color indicated the presence of amino acids.

#### Test for fixed oils and fats

Press small quantity of the petroleum ether extract between two filter paper. Oil stains on the paper indicated the presence of fixed oils.

Note: the results for the above experiments can be noted as follows.

- If the response to the test is high it can be noted as +++ which indicates that the particular group is present as the major class.
- If the response is average then note it as ++ indicates the presence in moderate quantity.
- If the response is very small then note it as + indicating the presence of only in traces.
- If no response is then negative.

#### In vitro methods employed in anti-diabetic studies<sup>10</sup>

##### Inhibition of alpha amylase enzyme

Total of 500 $\mu$ l of test samples and standard drug (100-1000 $\mu$ g/ml) were added to 500 $\mu$ l of containing alpha amylase (0.5mg/ml) solution and were incubated at 25 $^{\circ}$ C for 10 minutes. After these, 500ml of a 1% starch solution in 0.02M sodium phosphate buffer (pH6.9) was added to each test tube. The reaction mixture were then incubated at 25 $^{\circ}$ C for 10 minutes. The reaction was stopped with 1ml of 3,5-dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 minutes, cold to room temperature. The reaction mixture was then diluted after adding 10ml distilled water and absorbance was measured at 540nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle [11][12].

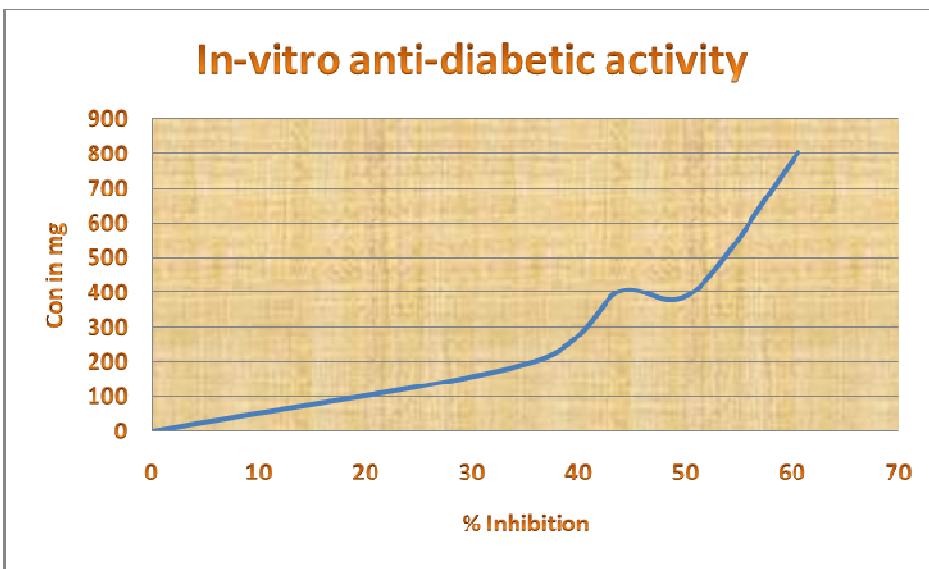
## RESULTS AND DISCUSSION

Table:1 Preliminary phytochemical screening of the seeds of *Heracleum candolleanum*

CLASS OF COMPOUND	TESTS PERFORMED	RESULTS
Carbohydrates	Molisch's test	---
	Fehlings test	---
Phenols	Phosphomolybdic acid test	+++
Flavanoids	Shinoda test	---
	lead acetate test	---
Tannins	Braemer's test	---
Sterols	Salkowski's test	---
Alkaloids	Dragendorf's test	---
Glycosides	Legals test	---
Saponins	Foam test	---
Anthraquinones	Borntragers test	+++
Amino acid test	Ninhydrin test	---
Fixed oils and fats		---

Table:2 In-vitro anti-diabetic activity of the seeds

Concentration in mg	Percentage Inhibition
200	35.92
400	43.66
4600	50.70
800	60.56



Graph:1 In-vitro anti-diabetic activity of the seeds

*Heracleum candolleanum* is a highly potential seeds in terms of chemical constituents and pharmacological activity. The chemical pattern of the methanolic extract of the plant shows that the seed is very rich in phenols and Anthraquinones.

## CONCLUSION

From the present study we conclude that the seeds of the plant *Heracleum candolleanum* are a highly potential seeds in terms of chemical constituents and pharmacological activity. Even though the plant is very rare and having wild origin it is being used by various traditional practitioners for various ailments.

The chemical pattern of the methanolic extract of the plant shows that the seed is very rich in phenols and anthraquinones. So we recommend further chemical phytochemical work in the plant because the identification of new chemical compounds from the plant will be emergence of new potent anti-diabetic molecules.

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