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Phytochemical Screening and *in vitro* antidiabetic activity of methanolic extract of fernandoa adenophylla

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Abstract
Diabetes Mellitus is a metabolic disorder characterized by hypoglycemia, resulting from absolute or relative deficiency of insulin. Worldwide about 220million people affected. Low-cost herbal treatment is recommended due to their lesser side effect. The aim of the current study was to determine the antidiabetic activity of methanolic leaf extract of *Fernandoa adenophylla* using alpha amylase inhibition assay. The phytoconstituents present in the plant extract showed an effect on alpha amylase. Hence the plant could be used to develop drugs against diabetic and also may be effective against other illness.

Keywords: phytochemical screening, I*n vitro* antidiabetic activity, hydro-alcoholic leaf extract, fernandoa adenophylla

Introduction
Diabetes mellitus (DM) is a chronic metabolic disorder, which often arises from deficiency in insulin secretion in the beta cells of pancreas. Over 415 million individuals with diabetes were reported in 2015 by the International Diabetes Federation. Also, higher prevalence, morbidity and mortality have been found in diabetic patients who suffer from complications of retinopathy, nephropathy, and peripheral neuropathy [1-3]. There are two types of diabetes. Type 1, insulin-dependent diabetes mellitus, characterizes with failure insulin secretion due to pancreatic β-cell destruction. People with Type 1 diabetes are always injected with insulin to keep alive. Whereas type 2 diabetes mellitus (T2DM), noninsulin-dependent diabetes mellitus, accounting for nearly 90% of all diabetic patients, features deficient insulin secretion and severe insulin resistance, concomitantly intolerance of glucose and changes in lipid and protein metabolism and in case of COVID it is also lethal in many patents [4-5].
Fernandoa adenophylla commonly known as Haplophragma adenophyllum which belongs to family Bignoniaceae. It is a flowering plant specifically native of India and Vietnam. It is also known as katsagon, marodphali, petthan, and Karen wood, it is up to 40m tall, branchless are 70cm in diameter, prop are absent. The flowers mostly remain closed in the day and open up at night. The fruit is long and twisted, hanging like snakes from the branches. *Fernandoa adenophylla* contains phytoconstituent such as triterpenoids (β-sitosterol, β-amyrin, ursolic acid, and oleanolic acid), napthaquinones and its derivatives (dilapachone, adenophyllone, peshwaraquinone, lapachol, and indadone) [6-8]. Various pharmacology activity has reported and assessed related to plant of Bignoniaceae family such activity are antioxidant, antimicrobial, antifungal, anti-TB, anti-inflamatory. Phytoconstituent extracted from plant has been effectively used for diseases known as sickle cell anemia, rheumatoid arthritis, piles, constipation, snake bite, skin disorder, heart diseases, gynecological disorders, malaria etc. Also there are used by Chakma tribe (Bangladesh) to treat haemorrhoids and constipation [9-12]. In Thai traditional medicine, leaves are employed to treat skin disorder. In traditional medicine it is used as massage oil ingredient to reduce muscular tension but sparingly.

Material and Methods
Collection and authentication of the plant
The plant part leaves and bark has been collected from the Fernandoa adenophylla tree. The plant specimen was authenticated from CSIR-NISCAIR.

Chemical
Commercial baker's yeast (Hi Media), metronidazole (Hi Media), dimethyl sulfoxide (DMSO) (Hi Media), solid DPPH (Hi Media), and ascorbic acid (Hi Media) and Ethanol (Merk).

Preparation of Plant Extract
The coarsely powder of leaves was placed in thimble chamber of the soxhlet apparatus. The extraction solvent methanol was heated in the bottom flask, vaporizes into the sample thimble, and condenses in the condenser and drip back. When the liquid content reached the siphon arm, the liquid contents emptied into the bottom flask again and the process was continued [13].
About 10 gm of sample powder has been weighed and extraction process was carried out by using 250ml of hydro alcohol in soxhlet apparatus for 48h. The extract was concentrated by evaporation for 8h and then dried. The concentrated and stored at room temperature before to phytochemical screening.

Phytochemical screening
The prepared extract of the plant was used to test various phytoconstituents present in them. Different chemical reagents were prepared and specific test, for specific phytochemicals was done. These various tests were qualitative and hence termed phytochemical screening. All chemicals and solvents were procured from Fisher Scientific, India, and were used without further purification. The tests were done by following standard procedures based on literature search [14-16].

1. Alkaloids
Evaporate the aqueous, alcoholic extracts separately. To residue, add dilute HCl, shake well and filter with filtrate, perform following test:

a. Mayer's test: 2-3 ml filtrate with few drop mayer's reagent gives ppt.
b. Wagner's test: 2-3 ml filtrate with few drop wagner's reagent gives reddish brown ppt.

2. Flavonoids
Shinoda test: To dry powder or extract, add 5ml 95% ethanol, few drops conc. Hcl and 0.5g magnesium turnings, pink colour observed.

3. Saponin
Foam test: shake the drug extract or dry powder vigorously with water. Persistent foam observed.

4. Glycoside
Keller- killianic test: to 2ml extract, add glacial acetic acid, one drop 5% $Fecl_3$ and Conc H_2So_4. Reddish brown color appears at junction of the two liquid layers and upper layer appear bluish green.

5. Steroid
Salkowski reaction: to 2ml of extract, add 2ml chloroform add 2ml Conc H_2So_4 shake well. Chloroform layer appears red acid layer shows greenish yellow fluorescence.

6. Tannins
0.5g extract was boiled in 20ml of water in a test tube and then filtered. Few drops of 0.1% ferric chloride was added and observed for brownish green or blue black color.

Methods for *in vitro* antidiabetic study
Determination of glucose uptake capacity by yeast cell. This assay has been performed according to the well-defined method of cirillo. Commercial baker's yeast was dissolved in distilled water to prepare 1% suspension. The suspension was kept overnight at room temperature (25°C). On the next day, yeast cell suspension was centrifuged at 4200rpm for 5 minutes. The process was repeated by the addition of distilled water to the pallet until a clear supernatant was obtained. Exactly 10 parts of the clear supernatant fluids were mixed with 90 parts of distilled water to get a 10% v/v suspension of the yeast cells [17].
About 1-5 mg w/v of plant extract was mixed with dimethyl sulfoxide (DMSO) till with various concentration (5, 10, and 25Mm) of 1ml of glucose solution and incubated for 10min at 37°C. To initiate the reaction, 100** of yeast suspension was poured in the mixture of glucose and extract, vortexed, and glucose was estimated by using a spectrophotometer (UV 5100B) at 520nm. Absorbance for the respective control was also recorded on the same wavelength. The percent increase in uptake was calculated by the formula.

% increase in glucose uptake = (Abs of control – Abs of sample) * 100 Abs. of control

Where control is the solution having all reagent except the test sample. Metronidazole was used as standard drug.

DPPH radical scavenging assay
Total antioxidant activity of plant extracts were estimated using stable DPPH radical scavenging assay. The assay was performed using ascorbic acid as reference standard [18-19]. The concentration required to reduce the initial DPPH radical concentration by 50% was calculated using the equation % Inhibition = Absorbance of control - Absorbance of sample/ Absorbance of control X100

Result and Discussion
Phytochemical analysis of the extract of the plant sample revealed the presence of saponins, flavonoids, tannins, alkaloids and sterols (table 1). This reveals that the secondary metabolites produced by the plant which can be extracted and utilized for the benefits of other living beings as they exhibit therapeutic and non-therapeutic properties. Antioxidant activity of the extract showed a dose dependent increase in the scavenging activity and exhibited a greater inhibition compared to activity of ascorbic acid and IC_{50} value was 81. 29 µg/mL (fig 1).

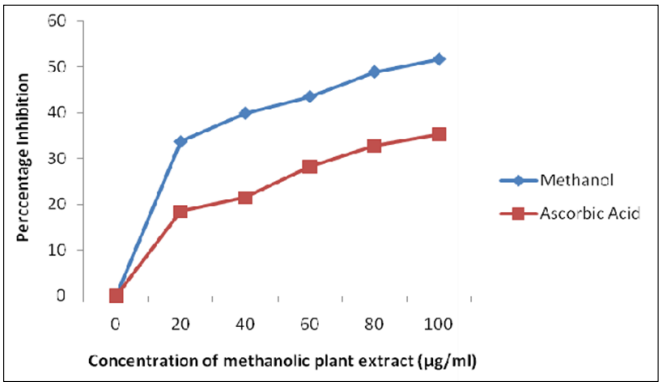

Fig 1: Antioxidant activity of the extract

Table 1: Phytochemical Screening of leave extract of *Fernandoa adenophylla*

Phytoconstituents	Leaves	Bark
Alkaloids	+	+
Mayer's test	-	-
Wagner's test	-	+
Flavonoids	+	-
Saponin	+	-
Glycoside	-	-
Steroid	+	-

Conclusion
Diabetes Mellitus is a metabolic disorder characterized by hypoglycemia, resulting from absolute or relative deficiency of insulin. Worldwide about 220million people affected. Low-cost herbal treatment is recommended due to their lesser side effect. The aim of the current study was to determine the antidiabetic activity of hydro-alcoholic leaf extracts of *Fernandoa adenophylla* using alpha amylase inhibition assay. The phytoconstituents present in the plant extract showed an effect on alpha amylase. Hence the plant could be used to develop drugs against diabetic and also may be effective against other illness.

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Conflict of Interest
The author declared no conflict of interest

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