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Evaluation of standardisation parameters, pharmacognostic study, preliminary phytochemical screening and *in vitro* antidiabetic activity of *Emblica officinalis* fruits as per WHO guidelines

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Abstract
Emblica officinal, medium size deciduous plant is available all over India and well known for ant diabetic activity. The qualitative Phytochemical screening procedure was performed on each extract. Phytochemical study reveals that carbohydrate, tannins, phenols, alkaloids, saponins was present in both the extracts. An attempt has been made to highlight this folk herbal medicine known for its antidiabetic property. In the present investigation, aqueous extract, and ethanolic extract of the fruits were made using hot extraction procedure using soxhlet apparatus, decoction, maceration through present study which will assist in the identification of fresh as well as dried crude samples of fruits anatomically and physicochemical. TLC finger printing and fluorescence analysis of powdered fruits has been conducted and reported. The Antidiabetic activity is conducted by enzyme inhibition (α-glycosidase) in *in vitro* method on each extract and ethanolic extract showed significant inhibition

Keywords: *Emblica officinalis*, ant diabetic activity, ethanolic extract, Phytochemical. α-glycosidase

1. Introduction
Plants used in traditional medicine have stood up to the test of time and contributed many novel compounds for preventive and curative medicine to modern science. India is sitting on a gold mine of well recorded and traditionally well practiced knowledge of herbal medicine. Specially, plants growing at high altitude in Himalayan pastures are time-honoured sources of health and general well-being of local inhabitants. As of today, Himalayan plants are a major contributor to the herbal pharmaceutical industry both of India and other countries. Plants growing at higher altitudes are subjected to an assault of diverse testing situations including higher doses of mutagenic UV-radiation, physiological drought, desiccation and strong winds. Plants interact with stressful environments by physiological adaptation and altering the biochemical profile of plant tissues and producing a spectrum of secondary metabolites. Secondary metabolites are of special interest to scientists because of their unique pharmacophores and medicinal properties [1]. Plant profile Amla is the medium size deciduous plant. It grows to the height of 8 -18 meters. It has a crooked trunk and spreading branches. Its flower is yellow greenish in colour. The fruit is spherical pale yellow with six vertical furrows. The mature fruits are hard and do not fall for the gentle touch. The average weight of the fruit is 60 -70 g. It has a gray bark and reddish wood. Its leaves are feathery, linear oblong in shape and smell like lemon. Its wood is hard in texture. It wraps and splits when exposed in the Sun or in the excessive heat [2].

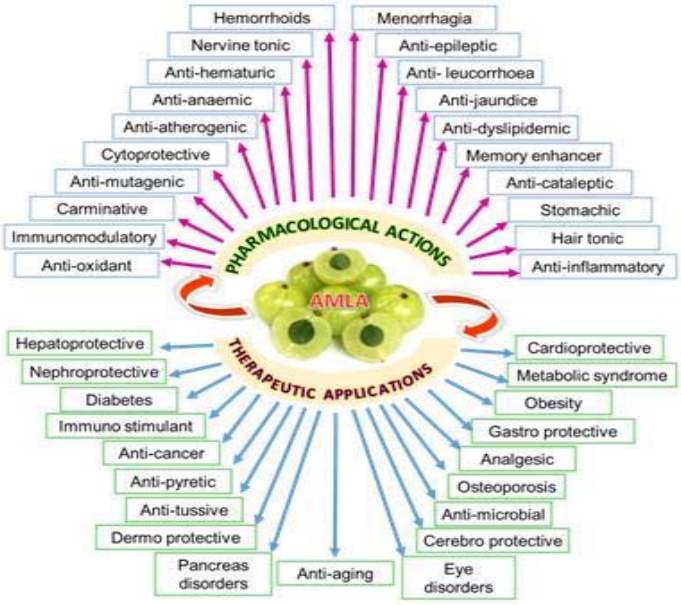
Fig 1: Fruits of *Emblica officinalis*

Table 1: Common Name for *Emblica officinalis* [3]

Languages	Traditional name
Hindi	Alma
English	Gooseberry, Embolic Myrobalan
Latin	*Emblica officinalis* Gaerte
Sanskrit	Aamalki, Dhatri Pacifies Tridoshas
Tamil	Nelli
Kannada	Nellaka
Telugu	Usirikaya
Malayalam	Nellimaram
Marathi	Amla
Bangla	Aamalki
Tibetan	Skyu-ru-ra
Portuguese	Mirabolano emblico
Malaysian	Popok Melaka
Nepalese	Amba
Chinese	An Mole
Italian	Mirabolano emblico
German	Amla

Table 2: Chemical constituents of Plant *Emblica officinalis* [4]

S. No	Chemical Constituents
1	Tannins
2	Alkaloids
3	Phenolic compounds
4	Amino acids
5	Carbohydrates
6	Vitamin C
7	Flavonoid
8	Ellagic acid
9	Chebulinic acid
10	Quercetin
11	Chebulagic acid
12	Emblicanin-A
13	Gallic acid
14	Emblicanin-B
15	Punigluconin
16	Pedunculagin
17	Citric acid
18	Ellagitannin
19	Trigallayl glucose
20	Pectin



Pharmacology and clinical studies of *Emblica officinalis*

2. Materials and Methods

2.1 Collection and authentication

Emblica officinalis fruits were collected from local market of Nalgonda, Nalgonda Dist., Telagana and India. All plant materials were collected from the months of December to January 2013-14.The plant material was identified and authenticated by Mr. Siddulu lecturer and head of botany department, Nagarjuna Government degree college, Nalgonda.

2.2 Chemicals and Reagents all

Reagents of analytical grade and highly pure.

2.3 Equipment requirement

UV Fluorescence Analysis cabinet (Secor India/Mumbai), Heating mantle (Sun Bim, India), Rotary Vacuum evaporator (Indosati, India), pH Meter (Elico Ltd/Model no.LI 120, Hyderabad), Pipettes & burettes (Borosil) has been used.

2.4 Morphological study

The drug was evaluated by its colour, odour, taste, size, shape and special features, like texture, touch, etc. evaluation was carried based on the morphological and sensory profiles of whole drug

2.5 UV Fluorescence analysis

Powdered fruits of *Emblica officinalis* were subjected to analysis under ultra violet light after treatment with various chemical and organic reagents. Three parameters were taken into account i.e., observation under long wave length U.V (365 nm), short wave length U.V (256nm) and normal day light. [5]

Table 3: UV fluorescence analysis of *Emblica officinalis*

Reagents	Long wave length			Short wave length			Day light		
	0 min	30 min	24 hrs	0 min	30 min	24 hrs	0 min	30 min	24 hrs
Con. H_2So_4	Black	Black	Black	Green	Light Green	Black	Brown	Light Green	Yellow
Con. HCL	Black	Black	Black	Light Green	Light Green	Green	Yellow	Light Yellow	Yellow
Pet. Ether	Black	Light yellow	Black	Yellow	Light Green	Green	Light Yellow	Light Yellow	Yellow
Ethanol	Black	Black	Black	Green	Green	Green	Light Yellow	Brown	Yellow
Methanol	Black	Black	Black	Green	Light Green	Green	Light Brown	Light Brown	Yellow
$Fecl_3$	Black	Black	Black	Black	Black	Black	Black	Black	Black
Benedicts	Black	Black	Black	Green	Green	Green	Black	Brown	Brown
Biuret	Black	Black	Black	Green	Black	Black	Brown	Brown	Brown
Dragendroffs	Black	Black	Black	Light Green	Light Green	Green	Light Brown	Light yellow	Violet
Mayer's	Black	Black	Black	Green	Green	Violet	Light Brown	Violet	Yellow
Barfoeds	Black	Black	Black	Green	Green	Green	Green	Yellow	Violet
Ninhydrin	Black	Black	Black	Green	Green	Green	Light Yellow	Violet	Yellow
Wagner's	Black	Black	Black	Black	Green	Green	orange	Brown	Yellow
Hager's	Black	Black	Black	Green	Green	Green	Green	Yellow	Violet
Picric acid	Black	Black	Black	Green	Green	Green	Black	Black	Light Green
Dil.NH_3	Green	Light Green	Black	Green	Green	Green	Brown	Light Green	Green
Dil. HCL	Black	Black	Black	Light Green	Light Green	Green	Yellow	Light Green	Yellow
Dil. H_2SO_4	Black	Black	Black	Green	Light Green		Light Yellow	Light Yellow	Yellow
$CHCl_3$	Black	Black	Black	Green	Green	Green	Brown	Brown	Yellow
Dil.HNO_3	Black	Black	Black	Green	Green	Green	Light Yellow	Light Yellow	Violet
Acetic acid	Black	Black	Black	Green	Green	Green	Light Yellow	Brown	Violet

2.6 Microscopic study [6]

Calcium oxalate Crystal **Fibers** **vascular bundles**

Fig 2: Microscopic characters of *Emblica officinalis*

2.7 Proximate analysis

Proximate analysis was carried out for the fruit powder of *Emblica officinalis* [7, 8, 9]

Table 4: Standardisation parameters of *Emblica officinalis* fruit powder

S. No	Parameters	%w/w
1	Total ash value	7.5%
2.	Acid insoluble ash	8%
3.	water-soluble ash	2%
4	Alcohol soluble extractive	20.8%
5	Water soluble extractive	31.2%
6	Moisture content	25.4%
7	Swelling index	7ml
8	Foaming index	100
9	Foreign matter	No foreign matter
10	True and bulk density	0.196g/ml 0.266g/ml
11	pH 1) Decoction 2) Soxhelation 3) Water maceration 4) alcohol maceration	 2.55 2.56 3.16 2.57

2.8 Limit test for iron

There has been not much intense colour observed than the standard iron solution hence the test passes for the iron. [10]

2.9 Extraction

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedure. [11]

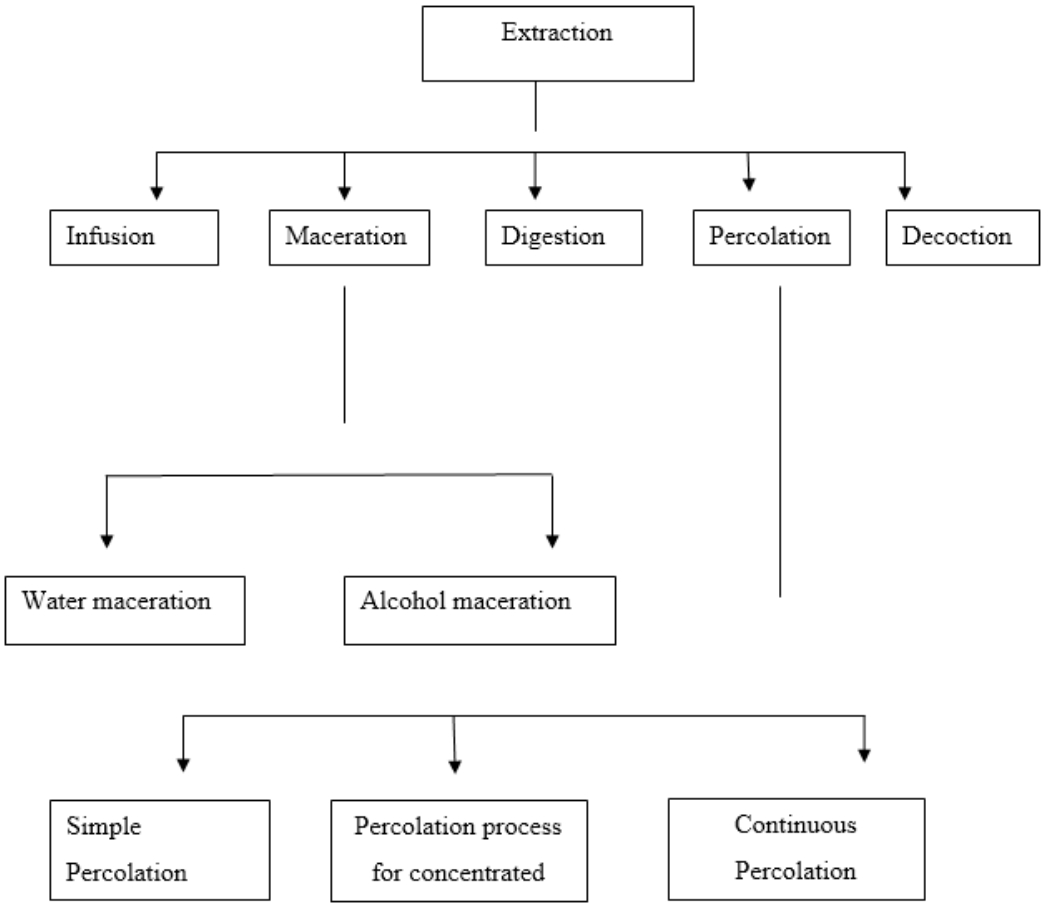


Fig 3: Flow chart of extraction procedures

a) Decoction

In this process, the crude drug (20 g) is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat-stable constituents. The starting ratio of crude drug to water (320ml) is fixed, e.g. 1:4 or 1:16; the

volume is then brought down to one-fourth its original volume by boiling during the extraction procedure [12].

b) Maceration

i) Alcohol maceration

In this process the drug (20 g) is placed with the whole of the alcohol (360 ml) in a closed vessel for 7 days. During this period shaking is done occasionally. After 7days the liquid is strained and marc is pressed.

ii) Water maceration

This method is similar to alcohol maceration where water is used instead of alcohol [13].

c) Continuous hot percolation process /soxhlet extraction

The drug (25 g) to be is packed in a paper cylinder made from a filter paper and it is placed in the body of soxhlet extractor. The alcohol (200 ml) is placed in the flask. The apparatus is fitted. The process of filling and emptying of the extractor is repeated until the drug is exhausted [13].

Table 5: Percentage yield of different extraction methods

S. No	Type of extraction	%yield (w/w)
1	Decoction(water)	60.95%
2	Alcohol maceration	56.25%
3	Water maceration	55.95%
4	soxhlet extraction(ethanol)	58.4%

2.10 Preliminary Phytochemical screening

After extractions the extracts were subjected to a vacuum rotary evaporator and concentrated extracts were obtained along with solvent recovery [14].

Table 6: Phytochemical screening of different extracts of *Emblica officinalis* fruit powder

Tests	Decoction	Alcohol maceration	Water maceration	soxhlet extraction
Carbohydrates				
Molisch test	+	+	+	+
Fehling's test	+	+	+	+
Barfoeds test	-	-	-	+
Benedict's test	+	+	+	+
Test for pentose's	-	-	-	-
Tannins				
Ferric chloride test	+	+	+	+
Chlorogenic acid test	-	-	-	-
Gelatin test	+	+	+	+
Saponins				
Froth formation test	+	+	+	+
Flavonoids				
Alkaline reagent test	-	-	-	-
Steroids and triterpenoids				
Liebermann bur chard test	+	+	+	+
Salkowski test	-	-	-	-
Sulphur powder test	+	+	+	+
Starch	-	-	-	-
Proteins				
Biuret test	-	-	-	-
Ninhydrin test	-	-	-	-
Alkaloids				
Dragendroffs test	+	+	+	+
Mayer's test	+	+	+	+
Wagner's test	+	+	+	+
Hager's test	+	+	+	+
Glycosides				
Bali jet test	-	-	-	-
Legal test	-	-	-	-
Test for hydroxy Anthraquinones	-	-	-	-
Phenols				
Lead acetate test	+	+	+	+

+ = indicates present

- = indicates absent

2.11 Test for vitamins [15]

Table 7: Test for vitamins to *Emblica officinalis* fruit extracts

Type of vitamin	Decoction	Alcohol maceration	Water maceration	soxhlet extraction
Vitamin D	+	+	+	+
Vitamin A	-	-	-	-
Vitamin C	+	+	+	+
Vitamin E	+	+	+	+
Vitamin K	-	-	-	-
Thiamine	+	+	+	+
Riboflavin	+	+	+	+
Pantothenic acid	+	+	+	+
Niacin	+	+	+	+

+ = indicates present
- = indicates absent

2.12 Vitamin C content estimation on *Emblica officinalis* [16]

Take 0.1g of extraction add 100ml of freshly boiled and cooled water and then add 25ml of 1M H_2SO_4.Add few drops of starch as an indicator. Then titrated with 0.05M I_2 colour change blue violet it's indicated presence of vitamin C

Table 8: Vitamin content estimation on *Emblica officinalis*

S.NO	Type of Extraction	%yield (w/w)
1	Decoction(water)	75%
2	Alcohol maceration	78%
3	Water maceration	75%
4	soxhlet extraction(ethanol)	82%

Fig 4: Vitamin C content estimation on *Emblica officinalis*

TLC method [17, 18]

2.13 Saponins

For detection of saponins required amount of the extract is dissolved in n-butanol and sample is applied on the plates using chloroform: GAA: methanol: water (64:32:12:8) as mobile phase using Anisaldehyde sulphuric acid reagent as visualising agent.

2.14 Phenols

For detection of Phenols required amount of the extract is dissolved in ether and sample is applied on the plates using acetic acid: chloroform (1:9) as mobile phase using vanillin-HCL as visualising agent.

2.15 Alkaloids

For detection of Alkaloids required amount of the extract is dissolved in chloroform and sample is applied on the plates using toluene: ethyl acetate: diethyl amine (7:2:1) as mobile phase using dragendroffs reagent as visualising agent.

2.16 Tannins

For detection of Tannins required amount of the extract is dissolved in chloroform and sample is applied on the plates using chloroform: Ethyl acetate: GAA(6:4:4) as mobile phase using vanilin-H_2SO_4 as visualising agent.

2.17 Carbohydrates

For detection of Carbohydrates of the extract is dissolved in chloroform and sample is applied on the plates using n-butanol: GAA: ether: water (9:6:3:1) as mobile phase. The observed spots are observed under long wavelength and short wavelength for fluorescence and resolved.

Table 9: R_f value of different compounds present in *Emblica officinalis* fruits

Name of Phytoconstituents	R_f Value of various Phytochemical constituents respective of extractions			
	Decoction	Water maceration	soxhelation	Alcohol maceration
Carbohydrates (A)	0.73	0.33	0.73	0.71
Tannins (B)	0.8	0.89	0.5	0.75
Phenols (C)	0.84	0.86	0.8	0.92
Saponins (D)	0.37	0.66	0.47	0.64
Alkaloids (E)	0.69	0.74	0.73	0.40

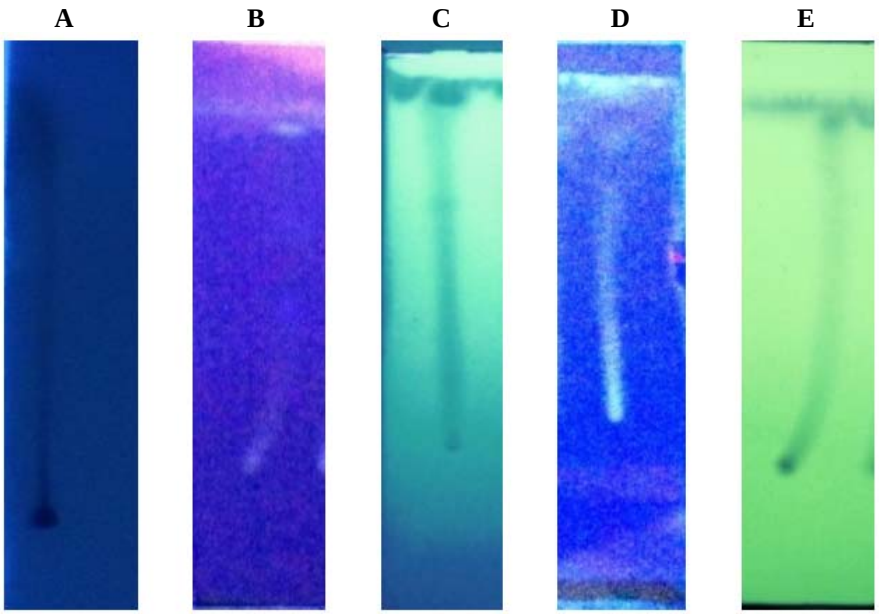


Fig 5: TLC finger printing of different extracts of *Emblica officinalis* fruits to estimate different phytoconstituents

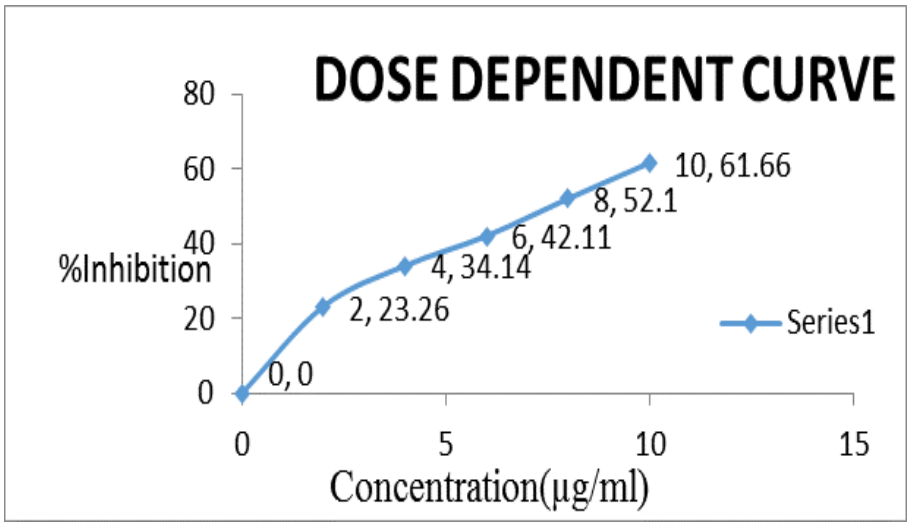


Fig 6: Dose dependent curve of soxhlet extract on enzyme inhibition

2.18 *In vitro* methods employed in antidiabetic studies
Inhibition of alpha-glycosidase enzyme [19]
The inhibitory activity was determined by incubating a solution of starch substrate (2% w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH8.0andvarious plant extract for 5 min at 37 °C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to it followed by incubation for 40 min at 35 °C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540 nm.

2.19 Calculation of Percentage inhibition (I %)
Percentage inhibition (I %) was calculated by

$$I\ \% = (Ac-As)/Ac\ X\ 100$$

Whereas,

A_c is the absorbance of the control and A_s is the absorbance of the sample

Table 10: % Inhibition of alpha-glycosidase enzyme by Amla fruit extract

S. No	Type of extract	% Inhibition
1	Water Maceration	20**%**
2	Decoction	30**%**
3	Alcohol Maceration	46.66**%**
4	Soxhelation(ethanol)	61.66**%**

Table 11: % *In vitro* Inhibition of alpha-glycosidase enzyme by soxhlation extract in dose dependent manner

S.NO	Concentration(µg/ml)	%inhibition
1	2	23.26
2	4	34.14
3	6	42.11
4	8	52.1
5	10	61.66

3. Discussion

According to the World Health Organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken . Macroscopic studies based on colour of fruits of plant *Emblica officinalis* a pepo, ovoid, glabrous, greenish brown to yellowish –brown with white linings; no odour and taste. In present study all the parameters are evaluated successfully as per auryveda pharmacopeia. Fluorescent studies of the fruits powder to wide range of colour changes at day light, UV-chamber (256 nm and 365 nm). The different extracts of the plant were subjected to the preliminary tests showed the presence of various constituents like Carbohydrates, saponins, tannins, phenols and alkaloids .The present finding reveals that *Emblica officinalis* fruits efficiently inhibits alpha-glycosidase enzyme *in vitro*. The alcohol macerated extract showed high inhibition and the range of inhibition ranges from 61.66-23.26%. The antidiabetic action of *Emblica officinalis* can also be attributed due to the presence of vitamin c on alpha-glucosidase inhibitory activity.

4. Conclusion

Diabetes is a serious metabolic disorder. Differences in social structure, psychic stress, obesity, hormonal imbalance and heredity are optimizing the growth of pandemic *Emblica officinalis*. is famous plant for its safe antidiabetic property. *Emblica officinalis* fruit powder extracts has been conducted *in vitro* antidiabetic activity .The plant showed significant inhibition activity, so further the compound isolation, purification and characterization which is responsible for inhibiting activity, has to be done for the usage of antidiabetic agent.

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