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Standardization and *In-vitro* anti-diabetic activity of *"Kovai Sarkarai"* - An analytical study.

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Abstract

Objective: The present study aimed to standardize *Kovai Sarkarai* and evaluate its anti-diabetic activity**. Method:** *Kovai Sarkarai* was prepared by removing the outer layer of the *Kovai* stem. The stem was ground with an appropriate quantity of water. Water was added in a ratio of 1:6 and the mixture was transferred to a porcelain container. Lemon juice was added, and the mixture was allowed to stand undisturbed for a period to facilitate sedimentation and obtain a clear supernatant. The clear liquid was filtered, and the filtrate was collected and dried to prepare *Kovai Sarkarai.* The prepared drug was analyzed for qualitative and quantitative parameters, including physicochemical evaluation, biochemical analysis, and HPTLC profiling, as per AYUSH testing protocols. The anti-diabetic activity of the drug was evaluated *in-vitro* using α-amylase and α-glucosidase inhibition assays. **Results:** The study revealed that *Kovai Sarkarai* was a brown-colored, tasteless powder. The pH of the formulation was found to be 6.58. Biochemical analysis indicated the presence of iron and alkaloids, while arsenic, lead, and mercury were absent. In the α-amylase inhibition assay, the extract exhibited a maximum inhibition of 63.58 ± 0.55% at a concentration of 500 µg/mL. In the α-glucosidase inhibition assay, the extract demonstrated a maximum inhibition of 44.72 ± 0.55% at a concentration of 500 µg/mL. **Conclusion:** The findings indicated that *Kovai Sarkarai* possessed a near-neutral pH and was free from heavy metal contaminants such as arsenic, lead, and mercury. The formulation exhibited significant *in- vitro* anti-diabetic activity through α-amylase and α-glucosidase inhibitory mechanisms. Therefore, the study concluded that *Kovai Sarkarai* possessed promising anti-diabetic potential and could be considered for further elaborate pharmacological and clinical investigations.

Keywords: *Kovai Sarkarai,* Anti-diabetic Activity, HPTLC, Siddha Medicine

Introduction

According to siddha fundamental philosophy, all the living and non living things in the world are formed by the combination of *Panjabootham*. Due to the fivefold combinations of these elements (*Pancheekaranam*) all 6 Suvai and 3 Humors like *Vatham*, *Pitham* and *Kabam* are formed. Dearangements in these vital humours cause diseases. *Siddhars* classified these diseases to 4448 types. One such condition is Diabetic Mellitus, which is on the rise due to alterations in lifestyle. Diabetes mellitus is a disease of inadequate control of blood levels of glucose.It has many subclassification, including type1, type 2,maturity –onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and steroid induced diabetes In 2019 ,diabetes was the direct cause of 1.5 million deaths and 48% of all death due to diabetes occurred before the age of 70 years . Another 46000 kidney disease deaths were caused by diabetes, and raised blood glucose causes around 20% of cardio vascular deaths. As per Indian Council of medical Research–India Diabetes (ICMR INDIAB) study published in 2023, the prevalence of diabetes is 10.1crores. "Food itself is a Medicine and Medicine itself is a Food" is the central tenet of Siddha. The Siddha medical system teaches us about the impact of our dietary choices on our overall health and well-being. In the Siddha system of medicine, diabetic mellitus is considered *Neerizhivu*. One of the polyherbal Siddha preparations stated in "Hakeeme P.M Abdullasayub -*Mega Nivarana Bothini* is *KOVAI SARKARI*, which is the subject of the current investigation. To far, no scientific research has been conducted to assess its therapeutic properties. The purpose of this study is to standardize and assess *in-vitro* anti-diabetic activity of *KOVAI SARKARI.*

Aim & Objectives

Aim

To standardize *kovai sarkarai* and evaluate its anti-diabetic activity.

Objectives

- To prepare *kovai sarkarai* with siddha literature *Mega Nivarana Bothini*
- To Standardize *kovai sarkarai (Coccinia grandis)* and evaluate its anti-diabetic activity through *in-vitro* study.

Materials and Methods

Indication: *Neerizhivu.*

Ingredients: Kovai stem, water, required amount of lemon juice.

Collection of kovai stem:

Kovai stem is collected from local farm in Kanyakumari

Preparation of kovai sarkkarai:`

- *Kovai Sarkarai* was prepared by removing the outer layer of the *Kovai* stem.
- The stem was ground with an appropriate quantity of water.
- Water was added in a ratio of 1:6 and the mixture was transferred to a porcelain container.
- Lemon juice was added, and the mixture was allowed to stand undisturbed for a period to facilitate sedimentation and obtain a clear supernatant.
- The clear liquid was filtered, and the filtrate was collected and dried to prepare *Kovai Sarkarai.* (Image:1)

KOVAI PLANT

KOVAI SARKARAI

KOVAI STEM

SEDIMENTED IS FILTERED

SOAKED IN WATER

DRY UNDER SUNLIGHT KEPT ASIDE

Images 1. Preparation of medicine

The following parameters were evaluated for kovai sarkarai:

Organoleptic characters:

- Colour
- Odour
- Taste

Colour:

The kovai sarkarai was taken into watch glasses and placed against white background in white tube light. It was observed for its Colour by naked eye.

Odour:

The kovai sarkarai was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste:

Small amount of *kovai sarkarai* was kept over the tip of the tongue.

Physiochemical analysis:

Physio-chemical studies of the plant drugs are necessary for scientific validation, as it helps in understanding the significance of physical and chemical properties of the substance being analysed in terms of their observed activities and especially for the determination of quality. This analysis includes the determination of ash value, Loss on drying a tie sample at 105°C, pH value and Extractive value. These were carried out as per

AYUSH protocol guidelines:

- pH
- Total ash
- Acid insoluble ash
- Loss on Drying
- Water soluble Extract
- Alcohol Soluble Extractive

pH:

A unit of measure that measures the acidity or alkalinity of a solution using a logarithmic scale with seven as neutral, where lower values are more acidic, and higher ones are more alkaline, is known as pH. The pH equals negative log10 of the hydrogen ion concentration (c), given in moles per litre pH = -log10 [H+] where, [H+] is the solution's hydrogen ion concentration, expressed in moles per litre. In an aqueous solution, the product of hydrogen ion concentration and hydroxyl ion concentration is constant, and the pH is equal to the negative logarithm of the concentration of hydrogen.

Total ash content:

2 to 3 g of *kovai sarkkarai* was weighed in the pre weighed and tared Gooch crucible was kept in the muffle furnace at a temperature not exceeding 450°C until it gets free from carbon. It is then cooled and weighed and the percentage of the total ash content was calculated with reference to the air dried drug.

Acid-insoluble ash:

The ash obtained from total ash is boiled with 25ml of dilute hydrochloric acid for 5 minutes and insoluble matters were collected in an ash less filter paper, washed with hot water and ignited to constant weight. Later the percentage of the acid insoluble ash content was calculated with reference to the air dried drug.

Percentage of acid-insoluble ash =

$$\frac{\text{Weight of the acid-insoluble residue}}{\text{Weight of the drug}} \times 100$$

Loss On Drying:

2gm of sample was accurately weighed (W,) in a tarred conical flask and kept in an oven for 4 hours specific temperature range of 100°C - 105°C. After cooled the sample in the tarred conical flask was gain weight (Wz).

Water Soluble Extractive:

Macerate 5g of the dried drug coarsely powdered, with 100ml of chloroform of the specified strength in a closed flask for 24 hrs. Shaking frequently during 6hrs and allowing to stands for 18hrs. Filter rapidly, taking precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a tarred flask bottomed shallow dish and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol soluble extractive with reference to the air dried drug.

Alcohol Soluble Extractive:

Macerate 5g of air-dried drug coarsely powdered, with 100ml of Ethanol of the specified strength in a closed flask for 24 hrs. Shaking frequently during 6hrs and allowing to stand for 18hrs. Filter rapidly, taking precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a tarred flask-bottomed shallow dish and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air dried drug.

Biochemical Analysis:

Solubility

A little amount of the sample is shaken well with distilled water.

Action of heat

A small amount of the sample is taken in a dry test tube and heated gently at first and then strong.

If White fumes evolved - Carbonates present.

If brown fumes evolved – Nitrate present.

Test for sodium

2 pinches of the substance is made into paste by using HCL and introduced into the blue flame of Bunsen burner .

If Yellow colour flame appeared – Sodium present.

Test for Fluoride and oxalate

2 ml of extract is added with 2ml of dil.acetic acid and 2ml calcium chloride solution and heated.

If Cloudy appearance present – Fluoride and oxalate present.

Test for Copper

2 ml of extract is added with excess of ammonia solution.

If blue coloured flame appeared – copper present.

Test for Iron

To the 2 ml of extract add 2ml of ammonium thiocyanate solution.

If mild red colour appear – Iron present.

Test for Aluminum, Zinc, Magnesium, Mercury, Arsenic

To 2ml of the extract sodium hydroxide solution is added in drops to excess.

If no characteristic changes present – absence of Aluminum, Zinc, Magnesium, Mercury, Arsenic.

Test for Reducing sugar

5.ml of Benedict's qualitatative solution is taken in a test tube and allowed to boil for 2 minūtes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes are noted.

If Brick red colour developed – Reducing sugar present.

Test for Alkaloids

2ml of extract is treated with 2 ml of picric acid

If Yellow colour developed – Alkaloids present.

HPTLC :

Sample Preparation for TLC:

One gram of sample was taken in 10 ml of chloroform, sonicated for 15 minutes and filtered.The filtrate was used for HPTLC analysis.

Thin-layer Chromatography Methodology:

Applied 10µl chloroform extract of *Kovai Sarkkarai* on TLC plate using Camag's ATS4applicator and developed by the mobile phase consists of Toluene: Ethyl acetate: Formic Acid(7.5:2.5:0.5, v/v/v) up to 9 cm distance. After development, the plate was photo documented using Camag's TLC Visualizer under UV 254 nm and UV 366 nm and then scanned using Camag's Scanner 4 at (D2 lamp/Absorption mode, Hg lamp/Fluorescent mode) finger print profiles of the extract were documented. Then the plate was dipped in vanillin-sulphuric acid reagent followed by heating at 105oC till development of colored spots. The plate was then photo documented in white light and scanned at 520 nm for finger print profile

Pharmacological activity:

In vitro anti-diabetic activity :

In-vitro Alpha Amylase Inhibition Study.

In-vitro Alpha Glucosidase Inhibition Study.

1.In-vitro Alpha Amylase Inhibition Study:

Method Adopted: The spectrophotometric assay method.

The enzyme α-amylase (0.5 U/ml) was prepared by mixing 3.24 mg of α-amylase in 100 ml of phosphate buffer (pH 6.9). Test Sample (KVS) was prepared in the serial dilution of the concentration ranges from 100,200,300,400 and 500 µg/ml using DD water. Acarbose 100 µg/ml used as a reference standard. About 600 µl of test sample were added to 30 µl of α-amylase enzyme solution and incubated at 37°C for 15 min. To this reaction mixture, 370 µl of substrate, 2-Chloro-4-Nitrophenyl-α-Maltotrioside ($CNPG_3$. 0.5 mg/ml) was added, mixed and for incubated 37°C for 10 min. Finally, absorbance was measured at 405 nm against blank in spectrophotometer. A control reaction was carried out without the test sample.

Percentage inhibition was calculated by the following formula.

Percentage inhibition

$$\% \text{ inhibition} = \frac{\text{Absorbance(control)} - \text{Absorbance(Test)}}{\text{Absorbance(control)}} \times 100$$

2. In-vitro α-Glucosidase Enzyme Inhibition Study:

Method Adopted: The spectrophotometric assay method.

Test Solution: Test Sample (KVS) was prepared in the serial dilution of the concentration ranges from 100,200,300,400 and 500 µg/ml using DD water.

PNPG (p-nitrophenyl-α-D -glucopyranoside**)**: 20 mM PNPG prepared by dissolving 603 mg PNPG in 100 ml of PBS

Enzyme: The α-glucosidase enzyme solution was prepared by dissolving 0.5 mg α-glucosidase in 10 ml phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin. About 10 µl of the test sample at vaying concentration along with Acarbose 100 µg/ml used as a reference standard were added to 250 µl of 20 mM p-nitrophenyl-α-D -glucopyranoside and 495 µl of 100 mM phosphate buffer (pH 7.0). It was pre-incubated at 37°C for 5 min and the reaction started by addition of 250 µl of the α-glucosidase enzyme solution prepared by 0.5 mg α-glucosidase in 10 ml phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin, after which it was

incubated at 37°C for exactly 15 min. 250 µl of phosphate buffer was added instead of enzyme for blank. The reaction was then stopped by addition of 1000 µl of 200 mM Na_2 CO_3 solution and the amount of p-nitrophenol released was measured by reading the absorbance of sample against a sample blank (containing PBS with no sample) at 405 nm using UV visible spectrophotometer.

Results

Organoleptic Characters

- Colour - Brown
- Odour - Unpleasant
- Taste - Tasteless

Biochemical Analysis:

• Solubility	-	Soluble
• Action of heat	-	Carbonate absent Nitrate absent
• Test for Sodium	-	Positive
• Test for Fluoride and oxalate	-	Fluoride absent Oxalate absent
• Test for Copper	-	Absent
• Test for Iron	-	Present
• Test for Aluminum	-	Absent
• Test for Zinc	-	Present
• Test for Magnesium	-	Present
• Test for Mercury	-	Absent
• Test for Arsenic	-	Absent
• Test for Reducing Sugar	-	Sugar absent
• Test for Alkaloids	-	Present

Physicochemical analysis

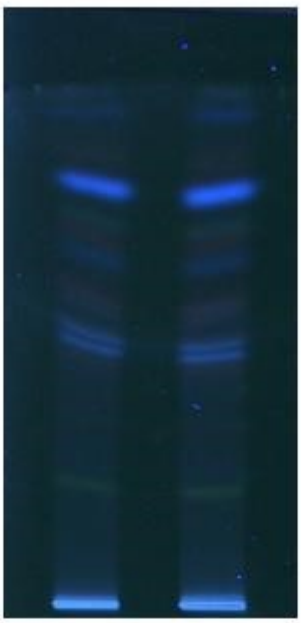
• pH	-	6.55
• LOD	-	10.08%
• Total ash	-	15.19%
• Acid insoluble ash	-	4.15%
• Water soluble extractive	-	26.97%
• Alcohol soluble extractive	-	13.42%

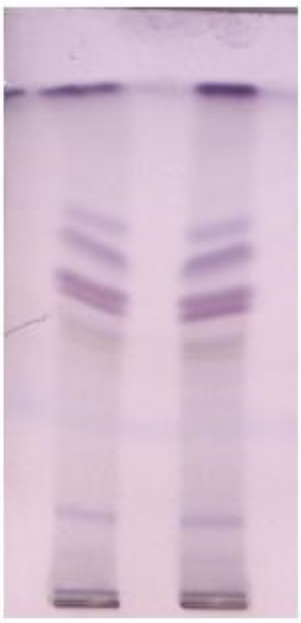
Phytochemicals:

- Saponin - Present
- Tannin - Absent
- Glycoside - Present

HPTLC:

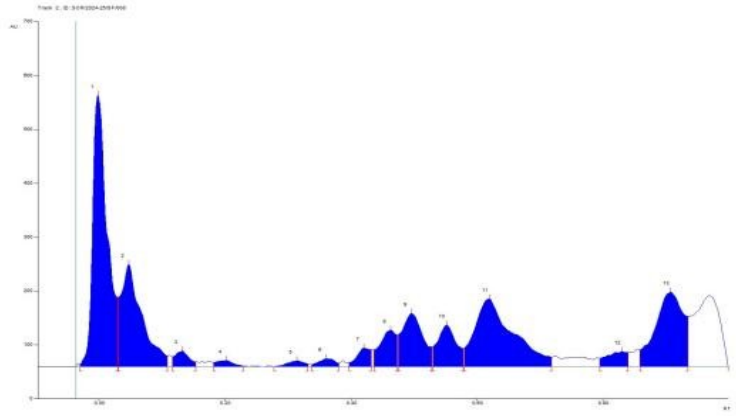
Image 2: TLC Photodocumentation





λ = 254 nm		λ = 366 nm		λ = 520 nm	
Color	**R_f value(s)**	**Color**	**R_f value(s)**	**Color**	**R_f value(s)**
Dark	0.05	Green	0.22	Pink	0.02
Dark	0.46	Blue	0.45	Pink	0.16
Dark	0.51	Blue	0.48	Yellow	0.48
Dark	0.57	Blue	0.49	Pink	0.56
Dark	0.66	Blue	0.65	Pink	0.59
		Red	0.69	Pink	0.66
		Green	0.72	Pink	0.72
		Blue	0.80		0.99
		Blue	0.95		

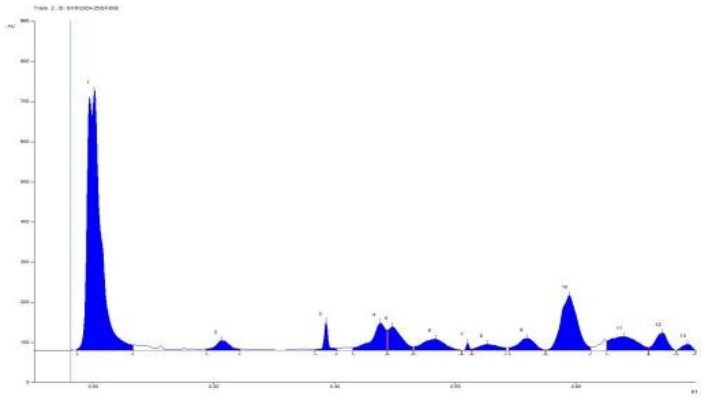
Image 3: HPTLC Chromatogram @ 254 nm:



Track 2, ID: SCRI2024-25/SF/008

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	3.9 AU	-0.00 Rf	503.3 AU	37.92 %	0.03 Rf	28.5 AU	11076.4 AU	27.47 %
2	0.03 Rf	128.7 AU	0.05 Rf	189.5 AU	14.28 %	0.11 Rf	19.8 AU	6032.6 AU	14.96 %
3	0.12 Rf	18.5 AU	0.13 Rf	28.5 AU	2.15 %	0.15 Rf	8.3 AU	618.1 AU	1.53 %
4	0.18 Rf	7.8 AU	0.20 Rf	11.4 AU	0.86 %	0.23 Rf	0.9 AU	292.8 AU	0.73 %
5	0.28 Rf	0.4 AU	0.31 Rf	10.6 AU	0.80 %	0.33 Rf	5.4 AU	267.1 AU	0.66 %
6	0.34 Rf	3.8 AU	0.36 Rf	14.6 AU	1.10 %	0.38 Rf	5.8 AU	367.9 AU	0.91 %
7	0.40 Rf	7.4 AU	0.42 Rf	34.3 AU	2.58 %	0.43 Rf	31.3 AU	710.4 AU	1.76 %
8	0.44 Rf	31.1 AU	0.46 Rf	67.5 AU	5.08 %	0.47 Rf	59.0 AU	1689.0 AU	4.19 %
9	0.48 Rf	59.4 AU	0.50 Rf	98.8 AU	7.44 %	0.53 Rf	36.5 AU	3120.5 AU	7.74 %
10	0.53 Rf	36.6 AU	0.55 Rf	77.3 AU	5.82 %	0.58 Rf	33.7 AU	2189.0 AU	5.43 %
11	0.58 Rf	34.4 AU	0.62 Rf	125.6 AU	9.46 %	0.72 Rf	17.2 AU	7253.6 AU	17.99 %
12	0.80 Rf	15.2 AU	0.83 Rf	27.7 AU	2.09 %	0.84 Rf	26.1 AU	874.5 AU	2.17 %
13	0.86 Rf	30.7 AU	0.91 Rf	138.1 AU	10.41 %	0.94 Rf	92.9 AU	5837.1 AU	14.47 %

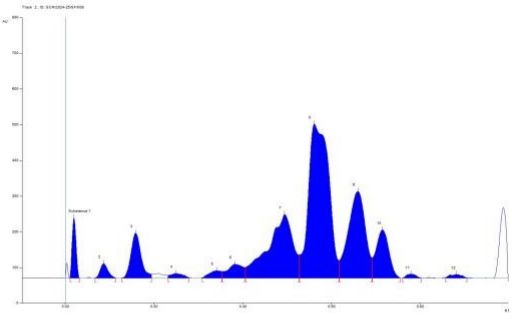
Image 4: HPTLC Chromatogram @ 366 nm:



Track 2, ID: SCRI2024-25/SF/008

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	2.5 AU	0.00 Rf	647.1 AU	54.59 %	0.07 Rf	13.1 AU	13741.3 AU	53.38 %
2	0.19 Rf	2.9 AU	0.21 Rf	23.6 AU	1.99 %	0.24 Rf	2.8 AU	533.6 AU	2.07 %
3	0.37 Rf	1.6 AU	0.39 Rf	70.4 AU	5.94 %	0.40 Rf	5.1 AU	451.0 AU	1.75 %
4	0.43 Rf	6.5 AU	0.48 Rf	67.5 AU	5.69 %	0.49 Rf	50.2 AU	1460.1 AU	5.67 %
5	0.49 Rf	50.2 AU	0.50 Rf	58.2 AU	4.91 %	0.53 Rf	9.2 AU	1237.2 AU	4.81 %
6	0.53 Rf	9.1 AU	0.57 Rf	27.8 AU	2.35 %	0.61 Rf	0.5 AU	998.3 AU	3.88 %
7	0.61 Rf	0.4 AU	0.62 Rf	17.6 AU	1.48 %	0.63 Rf	2.8 AU	86.5 AU	0.34 %
8	0.63 Rf	3.2 AU	0.65 Rf	15.2 AU	1.28 %	0.69 Rf	6.4 AU	470.9 AU	1.83 %
9	0.69 Rf	6.5 AU	0.72 Rf	29.7 AU	2.51 %	0.75 Rf	0.7 AU	789.3 AU	3.07 %
10	0.75 Rf	0.8 AU	0.79 Rf	136.8 AU	11.54 %	0.83 Rf	7.6 AU	3491.7 AU	13.57 %
11	0.85 Rf	23.6 AU	0.88 Rf	34.0 AU	2.87 %	0.92 Rf	7.2 AU	1427.5 AU	5.55 %
12	0.92 Rf	7.2 AU	0.95 Rf	42.6 AU	3.59 %	0.97 Rf	0.1 AU	824.1 AU	3.20 %
13	0.97 Rf	0.5 AU	0.99 Rf	15.0 AU	1.27 %	1.00 Rf	1.6 AU	228.7 AU	0.89 %

Image 5: HPTLC Chromatogram @ 520 nm:



Track 2, ID: SCRI2024-25/SF/008

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	2.6 AU	0.02 Rf	169.3 AU	11.92 %	0.03 Rf	1.0 AU	1425.6 AU	2.92 %
2	0.07 Rf	0.4 AU	0.09 Rf	40.8 AU	2.87 %	0.11 Rf	0.4 AU	688.0 AU	1.41 %
3	0.13 Rf	0.1 AU	0.16 Rf	126.2 AU	8.89 %	0.20 Rf	10.8 AU	2764.5 AU	5.67 %
4	0.23 Rf	7.9 AU	0.25 Rf	13.2 AU	0.93 %	0.28 Rf	2.0 AU	367.3 AU	0.75 %
5	0.31 Rf	1.5 AU	0.34 Rf	21.3 AU	1.50 %	0.35 Rf	18.6 AU	534.4 AU	1.10 %
6	0.35 Rf	18.6 AU	0.38 Rf	39.1 AU	2.75 %	0.41 Rf	29.9 AU	1296.9 AU	2.66 %
7	0.41 Rf	30.3 AU	0.50 Rf	178.7 AU	12.59 %	0.53 Rf	64.7 AU	9507.8 AU	19.50 %
8	0.53 Rf	64.9 AU	0.56 Rf	431.3 AU	30.37 %	0.62 Rf	49.7 AU	18966.6 AU	38.90 %
9	0.62 Rf	50.1 AU	0.66 Rf	242.9 AU	17.11 %	0.69 Rf	57.4 AU	8929.6 AU	18.32 %
10	0.69 Rf	57.5 AU	0.72 Rf	135.3 AU	9.53 %	0.76 Rf	0.1 AU	3755.3 AU	7.70 %
11	0.76 Rf	0.5 AU	0.78 Rf	11.4 AU	0.80 %	0.80 Rf	0.1 AU	237.8 AU	0.49 %
12	0.86 Rf	1.9 AU	0.88 Rf	10.5 AU	0.74 %	0.91 Rf	1.2 AU	279.1 AU	0.57 %

In-vitro anti-diabetic activity:

Table 1: Percentage inhibition of test drug KVS on Alpha Amylase enzyme Inhibition Study

Concentration (µg/ml)	% Inhibition of KVS
100 µg/ml	30.21 ± 2.17
200 µg/ml	38.77 ± 4.12
300 µg/ml	51.92 ± 2.57
400 µg/ml	57.62 ± 2.51
500 µg/ml	63.58 ± 0.55
Standard Acarbose	98.29 ± 0.77

Data are given as Mean ± SD (n=3)

IC50 Values for Alpha Amylase Enzyme inhibition by KVS and STD

Test Drug / Standard	IC50 Value of Alpha Amylase enzyme inhibition ± SD (µg /ml)
KVS	318.2 ± 4.421
Standard- Acarbose	28.48 ± 5.761

Data are given as Mean ± SD (n=3)

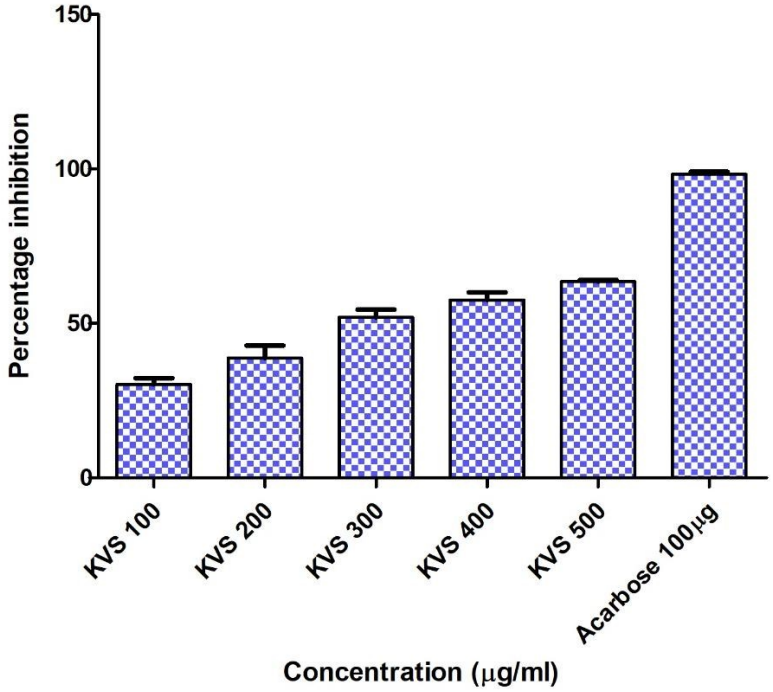


Table 2: Percentage inhibition of test drug KVS and STD on α-Glucosidase Enzyme Inhibition Study

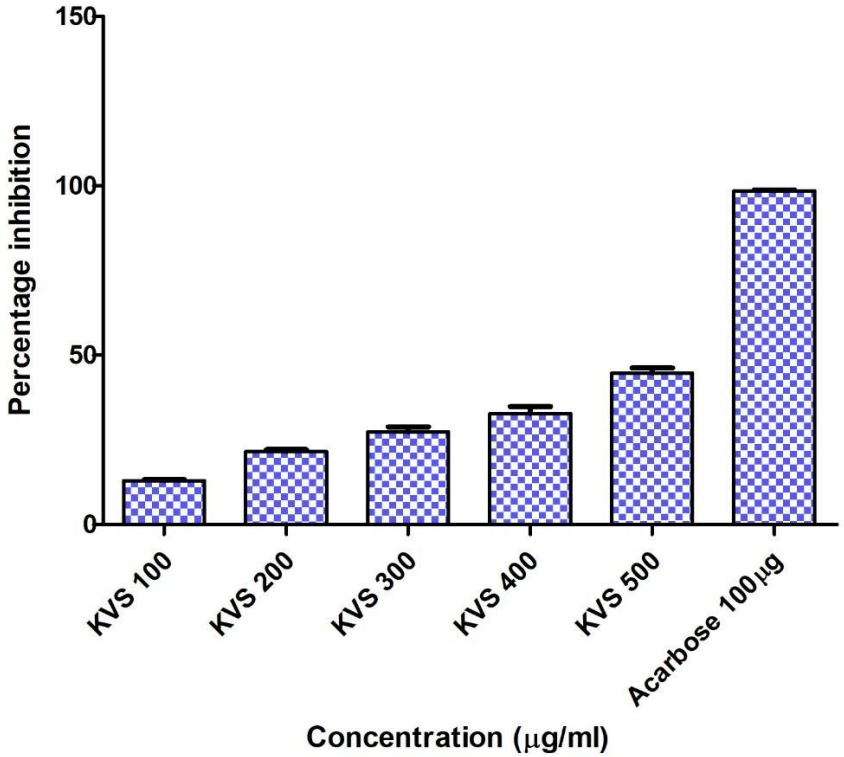
Concentration (µg/ml)	% Inhibition of KVS
100 µg/ml	12.91 ± 0.40
200 µg/ml	21.57 ± 0.49
300 µg/ml	27.49 ± 1.46
400 µg/ml	32.72 ± 2.11
500 µg/ml	44.72 ± 1.51
Standard- Acarbose	98.64 ± 0.22

Data are given as Mean ± SD (n=3)

IC50 Values for α-Glucosidase Enzyme Inhibition Assay by KVS and STD

Test Drug / Standard	IC50 Value of α-Glucosidase enzyme inhibition ± SD (µg /ml)
KVS	598.1 ± 17.57
Standard- Acarbose	33.51 ± 5.688

Data are given as Mean ± SD (n=3)



Observation

It was observed from the results of the present investigation that the formulation KVS shown promising alpha amylase enzyme inhibition potential with the maximum inhibition of about 63.58 ± 0.55% and the corresponding IC_{50} is 318.2 ± 4.421 µg /ml. Standard acarbose exhibited significant inhibition in alpha amylase enzyme with the maximum inhibition of about 98.29 ± 0.77 % and the corresponding IC_{50} is 28.48 ± 5.761 µg /ml.

It was observed from the results of the present investigation that the formulation KVS shown promising glucosidase enzyme inhibition potential with the maximum inhibition of about 44.72 ± 1.51% and the corresponding IC_{50} is 598.1 ± 17.57 µg /ml. Standard acarbose exhibited significant inhibition in alpha glucosidase enzyme with the maximum inhibition of about 98.64 ± 0.22 % and the corresponding IC_{50} is 33.51 ± 5.68 µg /ml.

Discussion

- *Kovai Sarkarai* is an brown olour,tasteless powder form of medicine.pH value 6.58.
- Biochemical analysis shows the presence of iron, alkaloids and absence of arsenic, lead, and mercury in this *Kovai Sarkarai.* It indicates that this drug was safe to consume.
- HPTLC analysis of *kovai sarkarai* at λ=254nm reveals the presene of 13 prominent peaks.Rf values of the peaks ranges from 0.03Rf to 0.86Rf.
- HPTLC analysis of *kovai sarkarai* at λ=366nm reveals the presene of 13 prominent peaks.Rf values of the peaks ranges from 0.03Rf to 0.97Rf.
- HPTLC analysis of *kovai sarkarai* at λ=520nm reveals the presene of 12 prominent peaks.Rf values of the peaks ranges from 0.01Rf to 0.86Rf.
- Alpha-amylase inhibition activity, the extract showed maximum percentage inhibition of 63.58± 0.55% at 500µg concentration
- Alpha-glcosidase inhibition activity, the extract showed maximum percentage inhibition of 44.72 ±0.55% at 500µg concentration

Conclusion

The findings indicated that *Kovai Sarkarai* possessed a near-neutral pH and was free from heavy metal contaminants such as arsenic, lead, and mercury. The formulation exhibited significant *in- vitro* anti-diabetic activity through α-amylase and α-glucosidase inhibitory mechanisms. Therefore, the study concluded that *Kovai Sarkarai* possessed promising anti-diabetic potential and could be considered for further elaborate pharmacological and clinical investigations.

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