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EXPLORING THE THERAPEUTIC POTENTIAL AND NUTRITIONAL PROPERTIES OF 'KARUPPU KAVUNI' VARIETY RICE OF TAMIL NADU

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ABSTRACT

Kavuni rice was considered to be best nutrition supplement since 400BC as it cures gastritis, peptic ulcer and also enhances blood circulation and strengthens the body. The target of this study is to reveal the other therapeutic properties of Karuppu Kavuni arisi through phytochemical screening and *in-vitro* assay. Karuppu Kavuni was extracted using Ethanol and the crude extract was analysed using GC-MS technique to determine its constituents. Antioxidant assay was carried out by Nitric oxide radical scavenging method and reducing power assay. Arthritic activity was also performed by Egg albumin denaturation method and inhibition of α-amylase and α-glucosidase activity was carried out to determine the Anti-diabetic potential of the rice. The percentage inhibition of the extract was found to be higher in 1000µg/ml compared to other concentration from the *in-vitro* assays. Therefore, it is reflected as the best Antioxidant, which is Anti-diabetic, reduces inflammation and prevents Arthritis.

KEYWORDS: ***Karuppu Kavuni arisi, GC-MS, Antioxidant assay, Anti-Arthritic activity, Anti-diabetic potential.***

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INTRODUCTION

Rice which is known as the prince among grains, belongs to the family Poaceae. Most of the world's population depend on rice because of its energy, fibre and nutritional benefits [1-2]. It is the second most consumed grain after wheat and a rich source of antioxidants. There are many traditional varieties of rice which are used to cure diseases [3]. In India, the traditional rice varieties were used for their medicinal value from Chakra Samihita period (400-700 BC) i.e. *Laicha* rice of Chhattisgarh and *Navara* rice from Kerala were used to cure gastritis and peptic ulcer. The *Bhatmoori* from Tamilnadu was used for curing anaemia and to enhance blood circulation in women during childbirth as it contains folic acid. The *Parmai-Sal, Kabiraj-Sal* from West Bengal had nutritive properties to strengthen the body [1]. Carbohydrates, fatty acids, proteins are the major components of rice mentioned above. But karuppu Kavuni, a traditional rice variety of Tamil Nadu is known for its nutritive properties and it is considered to be the best Antioxidant, Anti-arthritic and Anti-diabetic among other rice varieties. Kavuni grains are brown-black in colour which were reported earlier to have reduced levels of total soluble sugar, low fat content, increased protein content, high levels of phenolic acid, flavonoids, carotenoids and minerals like iron, manganese, zinc, copper, sodium, potassium, magnesium [4-8]. Karuppu Kavuni is one among the grains which farmers love[9]. If the colour of the pericarp of karuppu Kavuni grains be dark, it will have higher levels of polyphenols. During milling, the concentration of these compounds might be reduced [10]. Nowadays, consumption of polished white rice is more than traditional rice varieties which is the reason for clinical problems like high insulin resistance, dyslipidaemia [4] and it also cause oxidative stress, which shows the presence of Reactive Oxygen Species (ROS) and free radicals in excess which result in Alzheimer disease, Parkinsonism Cancer, Arthritis, Atherosclerosis, neuronal disorders. This situation occurs when there is an imbalance between the amounts of ROS produced in the body to that of natural antioxidants [11]. Whereas, Diabetes Mellitus (DM) is caused due to elevation of blood sugar level causing hyperglycaemic state in type II DM. This is due to hydrolysis of starch in food by pancreatic α-amylase and increased glucose uptake by α-glucosidase at intestinal level[12]. Also, Arthritis is caused by protein denaturation. Therefore, the intake of traditional rice variety like Kavuni on daily basis might improve one's health status. Although, not many studies have revealed the nutritional and therapeutic properties of traditional rice varieties[4]. This study unravels the therapeutic properties of Karuppu Kavuniarisi (*Oryzasataiva L.*), a traditional rice variety of Tamil Nadu.

MATERIALS AND METHODS

Collection of materials

The karuppu Kavuni arisi was purchased from Good Food organic shop, Tambaram Sanatorium, Chennai, Tamil Nadu, India. It was authenticated by V. Chelladurai, Former Research Officer – Botany (Scientist-C), Central Council for Research in Ayurveda & Siddha, Government of India.

Preparation of extract

The grains were checked for dust particles and stones. Then they were ground to powder form and was extracted with ethanol as mentioned in Suvarna Rani Ch (2013) by cold maceration. About 20 g of sample in 60 ml of ethanol was added i.e. in the ratio of 1:3 and it was incubated for 96 hours. Then the supernatant layer was collected and concentrated using water bath at low temperature like 40°C - 50°C[13]. This extract was used for phytochemical screening and other *in-vitro* studies.

Phytochemical screening and gc-ms analysis

The phytochemical screening was done using standard chemical tests methods [14-15]. And, the Gas chromatography – Mass spectroscopy (GC-MS) analysis was also done on the Ethanolic extract of karuppu Kavuni at IIT Madras (Reference No:GCMS 19/04/2017053117).

Anti-oxidant activity

Assay of Nitric oxide Radical Scavenging

- The method given in Arun KP, Brindha P (2014) was followed to determine the radical scavenging activity of the Ethanolic extract.
- It was determined by adding 400µL of 100mM sodium nitroprusside, 100µL of Phosphate Buffered Saline (PBS, pH - 7.4) and 100µL of different concentration (31.25µg/ml - 1000 µg/ml) of the Ethanolic extract.
- This was kept for incubation at 25°C for 150 minutes.
- To 0.5 mL of above solution, 0.5 mL of Gries's reagent was added.
- This was incubated at room temperature for 30 minutes, and finally absorbance was observed at 540 nm[16].
- Ascorbic acid was used as the referencedrug and treated similarly for the determination of absorbance.
- The method was performed in triplicates, and their percentage inhibition was calculated by using the formula given below and the values are expressed as mean ±standard deviation.

$$\%\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Reducing Power Assay

- Reducing Power assay procedure was carried out following Deepa S (2017) method.
- 1 ml of extract of various concentrations (31.25µg/m l - 1000 µg/ml) was mixed with 2.5 ml of Phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of Potassium Ferric Cyanide (1%) was kept at 50°C in water bath for 20 minutes.
- After the incubation period, to this reaction mixture, 2.5 ml of Trichlororacetic acid was added dropwise and centrifuged by fixing at 650 rpm for about 10 minutes.

- From the supernatant layer of about 2.5ml was taken and 2.5ml of distilled water was added and 0.5ml of Ferric Chloride (0.1%) was added and absorbance was measured at 700 nm[17].
- Ascorbic acid in the concentrations of 31.25µg/m l - 1000 µg/ml was used as the reference drug and treated similarly for the determination of absorbance.
- The method was performed in triplicates, and their percentage inhibition was calculated by using the formula given below and the values are expressed as mean ±standard deviation.

$$\%\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Anti-arthritic activity

Egg Albumin Denaturation Method

- The arthritic activity was performed as mentioned in Rahman et al. (2015) [18].The reaction mixture consisted of 0.2 mL of egg albumin, 2.8 mL of phosphate-buffered saline (PBS, pH6.4) and 2 mL of varying concentrations 31.25µg/m l - 1000 µg/ml) of the extract.
- The mixtures were incubated at 37 ± 2°C in incubator for 15 minutes and then heated at 70°C for five minutes.
- After cooling, their absorbance was measured at 660 nm by using the vehicle as a blank [18]. Diclofenac sodium in the concentrations of was used as the reference drug and treated similarly for the determination of absorbance.
- The method was performed in triplicates, and their percentage inhibition was calculated by using the formula given below and the values are expressed as mean ±standard deviation.

$$\%\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Anti-diabetic activity

α- Glucosidase inhibitory activity

- The α-glucosidase enzyme was freshly extracted for inhibitory assay based on the procedure mentioned in Agu RC. And Palmer GH. (1997).[19]
- The enzyme α-glucosidase inhibitory activity was performed according to Ramprasad R.et al (2016).[20] method.
- It was done by mixing α-glucosidase enzyme 0.5ml with 1 ml of various concentrations (31.25µg/ml - 1000 µg/ml) of extract.
- Then 3mM Sucrose solution 0.5ml was added as a substrate.
- This reaction mixture was incubated at 37°C for 30 minutes and the reaction was terminated by addition of 2 mL of sodium carbonate.
- The anti-diabeticactivity was determined by measuring at 400 nm[20].
- Acarbose in the concentrations of 31.25µg/ml - 1000 µg/mlwas used as the reference drug and treated similarly for the determination of absorbance.
- The method was performed in triplicates, and their percentage inhibition was calculated by using the formula given below and the values are expressed as mean ±standard deviation.

$$\%\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

α-Amylase inhibitory activity

- The wheat alpha amylase was extracted freshly and amylase inhibitory assay was done as laid down by Sindhu. S. Nair et al (2013) [21].
- The sample mixture had 200µl of 0.02M sodium phosphate buffer, 20µl of enzyme and Kavuni extracts in concentration range 31.25µg/ml - 1000 µg/ml, 200µl of starch and incubated for 10 minutes at room temperature.
- The reaction was terminated with the addition of 400µl di-nitrosalicylic acid reagent and placed in boiling water bath for 5 minutes.
- After heating it was cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm.
- Acarbose in the concentrations of 31.25µg/ml - 1000 µg/mlwas used as the reference drug and treated similarly for the determination of absorbance[21].
- The method was performed in triplicates, and their percentage inhibition was calculated by using the formula given below and values are expressed as mean ±standard deviation.

$$\%\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

All the tests were performed in triplicate and values are expressed as mean ± standard Deviation. The mean value and the standard deviation were calculated using a online software named miniWebtool. Available from/: https://www.miniwebtool.com. By using the formula below it was calculated:

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \bar{x})^2}$$

Where:
s = sample standard deviation
x1, ..., xN = the sample data set
x̄ = mean value of the sample data set
N = size of the sample data set

RESULTS AND DISCUSSION

The coloured rice variety like Karuppu Kavuni is a rich source of vitamins and minerals like iron, manganese, zinc, copper, sodium, potassium , magnesium therefore it was used in various stages of life in order to gain strength and prevent diseases like anaemia, diabetes, arthritis, etc. and also used to improve blood circulation and thereby helpful in preventing disease[22]. Even though various synthetic antioxidants, anti-diabetic, anti-arthritic drugs, etc. are available for the diseases happening, the side effects produced by them are more. Hence, we need a more potent and less toxic agent which reduces oxidative stress, diabetes, arthritis and also cures other diseases [11]. Accordingly, Karuppu Kavuni rice is considered to be a good Nutraceutical from the literature above.. In this study, it will been proven that it can be used to prevent disorder like diabetes, arthritis, stress disorders. Below are the results which show it is a good replacement for modern methods of healing.

Phytochemical screening and gas chromatography – mass spectrometry (gc-ms) analysis

The Ethanolic extract of Karuppu Kavuni was tested for the presence or the absence of Alkaloids, Anthocyanin, Flavonoids, Anthraquinones, Coumarins, Phenols, Proteins, Carbohydrates, Tannins, Amino acids, Steroids, Glycosides, Terpenoids, and Saponins. The constituents found to be present through chemical tests are given in Table 2. Also the GC-MS analysis was done for the same extract and the constituents identified are displayed in Table 1 and Graph 1 given below. These constituents present in the rice extract are responsible for the results obtained in the *in-vitro* antioxidant assay, anti-diabetic assay and in anti-arthritic assay.

Table 1
Components identified from GC-MS analysis of Extract of karuppu Kavuni arisi

S.NO	RETENTION TIME (minutes)	METABOLITES	RELATIVE CONTENT (%)
1	15.23	3-Buten-1-ol, 1-[napthyl]-	3.2
2	16.23	Trioxsalen	9.2
3	17.88	2,6,10-Dodecatrienoic acid, 3,7,11-trimethyl-, ethyl ester, (ZZ)-	9.9
4	18.77	4,6-dimethyl-2-oxo-1-p-tolyl-1,2-dihydro-pyridine-3-carboxylic acid amide	3.2
5	20.3	5H-1,2,4-Triazolo[3,4-a][2]benzazepine,7-methyl-5-spirocyclohexane-	67
6	19.87	Furan-2-carbohydrazide, N2-(3-indolylmethylene)	27.6
7	13.17	Glucose	3.7
8	14.95	d-Mannose	4.5

Graph 1
Chromatograms of GC-MS analysis of Extract of karuppu Kavuni arisi

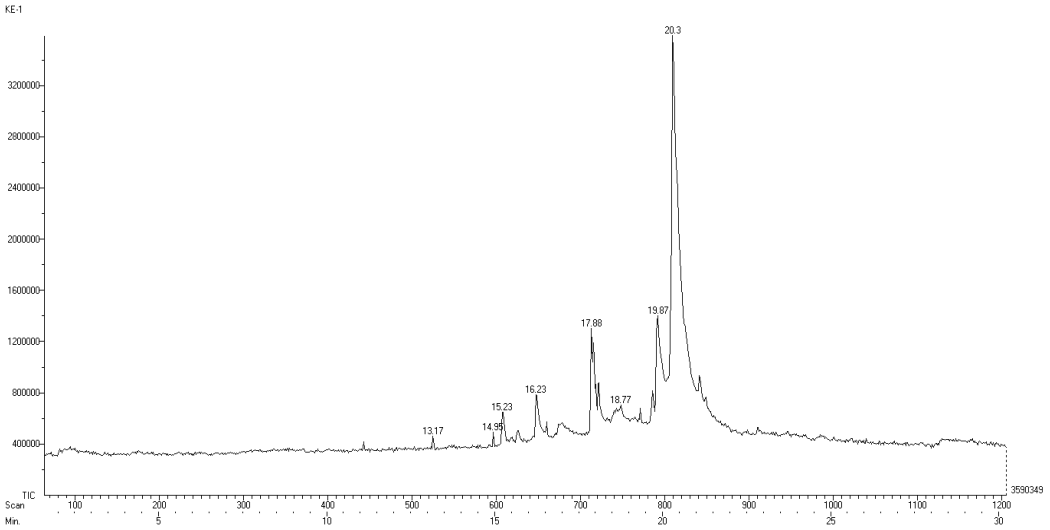


Table 2
Results Phytochemical analysis of Extract of KaruppuKavuni arisi

PHYTOCHEMICAL CONSTITUENTS	ETHANOLIC EXTRACT
Alkaloids	-
Anthocyanin	++
Flavonoids	++
Anthraquinones	-
Coumarins	+
Phenols	++
Proteins	-
Carbohydrates	++
Tannins	-
Amino acids	+
Steroids	-
Glycosides	++
Terpenoids	-
Saponins	-

(++) - Highly present, (+) - Slightly present, (-) - Not present

Antioxidant activity

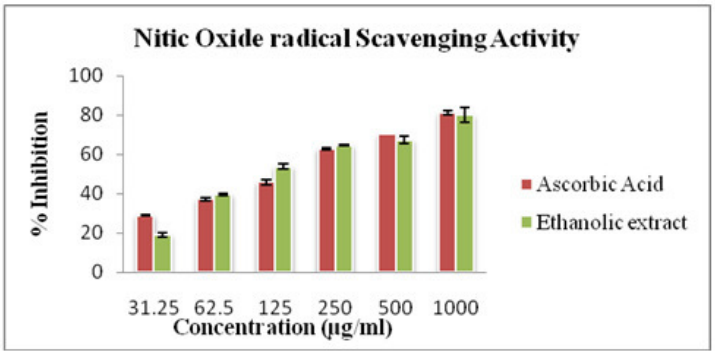
Assay of nitric oxide radical scavenging

The presence of 5H-1,2,4-Triazolo[3,4-a][2]benzazepine,7-methyl-5-spirocyclohexane-, Furan-2-carbohydrazide, N2-(3-indolylmethylene in abundance in the extract is responsible for the free radical scavenging property of the extract. The results shown by the extract is in dose dependent manner i.e. higher the concentration, increased percentage inhibition. The maximum free radical scavenging was observed at 1000µg/ml, with percentage inhibition of 80.07±3.54% while standard drug sowed 81.2±1.03%. In comparison with standard drug, it is revealed that it's a potent antioxidant. The percentage inhibition of other concentrations is listed in Table 3 and Graph 2.

Table 3
Results of Nitric oxide radical scavenging assay of Extract of karuppu Kavuni arisi

CONCENTRATION (µg/ml)	ASCORBIC ACID (Mean±SD)	ETHANOLIC EXTRACT (Mean±SD)
31.25	28.89±0.35	19.11±1.15
62.5	37.18±0.84	39.67±0.56
125	45.92±1.41	53.81±1.45
250	62.76±0.40	64.52±0.17
500	70.28±0.05	67.29±1.79
1000	81.2±1.03	80.07±3.54

Graph 2
Representations of percentage inhibition of Extract of karuppu Kavuni arisi



Reducing Power Assay

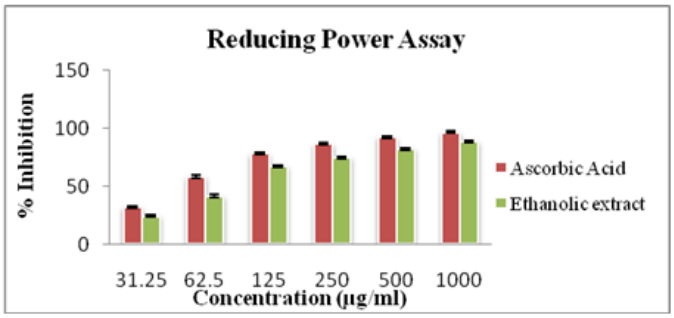
The reducing power assay reflects the exact antioxidant potential of a compound. Because, it is said that if an extract has good reducing power it will reduce the level of oxidized intermediates of lipid peroxidation process and pretend to be primary and secondary antioxidant. Here, the percentage inhibition of Kavuni extract was found to be 88.54% (1000µg/ml), whereas ascorbic acid was found to be 96.53% at 1000µg/ml concentration which is visible in Table 4 and Graph 3.This shows that

Kavuni can reduce oxidative stress and other related diseases at a greater level.

Table 4
Results of reducing power assay of Extract of karuppu Kavuni arisi

CONCENTRATION (µg/ml)	ASCORBIC ACID (Mean±SD)	ETHANOLIC EXTRACT (Mean±SD)
31.25	31.98±0.31	24.1±0.53
62.5	58.32±0.008	41.65±0.01
125	78.04±0.33	67.38±0.44
250	86.66±0.02	74.99±0.01
500	92.52±0.30	82.3±0.24
1000	96.53±0.22	88.54±0.25

Graph 3
Representations of percentage inhibition of Extract of karuppu Kavuni arisi



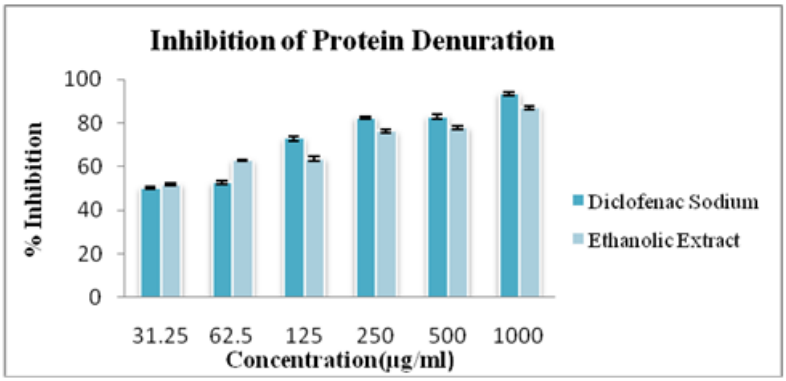
Anti-arthritic activity
Egg albumin denaturation method

From the reports of GC-MS analysis and antioxidant assays, the Ethanolic extract of Karuppu Kavuni was further tested for anti-arthritic activity. Protein denaturation is the main cause of arthritis which occurs due to disturbance between hydrogen bonds and hydrophobic interactions due to heat. Therefore, the rice extract was used in egg albumin denaturation method to find out if it can inhibit denaturation of proteins. It is determined that the extract inhibited 87.13% of protein denaturation when compared to Diclofenac sodium which showed 93.54% inhibition at 1000µg/ml, which can be viewed in Table 5, Graph 4.

Table 5
Results of Egg Albumin Denaturation Method of Extract of karuppu Kavuni arisi

CONCENTRATION (µg/ml)	DICLOFENAC SODIUM (Mean±SD)	ETHANOLIC EXTRACT (Mean±SD)
31.25	50.32±0.59	51.94±0.56
62.5	52.68±0.68	62.87±0.10
125	72.98±1.12	63.69±1.01
250	82.45±0.64	76.21±0.68
500	82.94±0.95	77.92±1.01
1000	93.54±0.65	87.13±0.75

Graph 4
Representations of percentage inhibition of Extract of karuppu Kavuni arisi



Anti-diabetic activity

A-glucosidase inhibitory activity

It is a known fact that, karuppu Kavuni is widely known for its anti-diabetic property from the literature, here are the results that prove it is a potent anti-diabetic. When a compound is known to inhibit alpha-glucosidase then it hinders the processing of carbohydrates and reduces the assimilation. Thereby, decreasing postprandial (PP) blood glucose levels in the blood after meal consumption. Similarly, the karuppu Kavuni extract showed 71.20% inhibition of α-glucosidase when compared to standard drug (Acarbose) which showed 73.10% inhibition at 1000µg/ml concentration. The results are given in Table 6 and Graph 5.

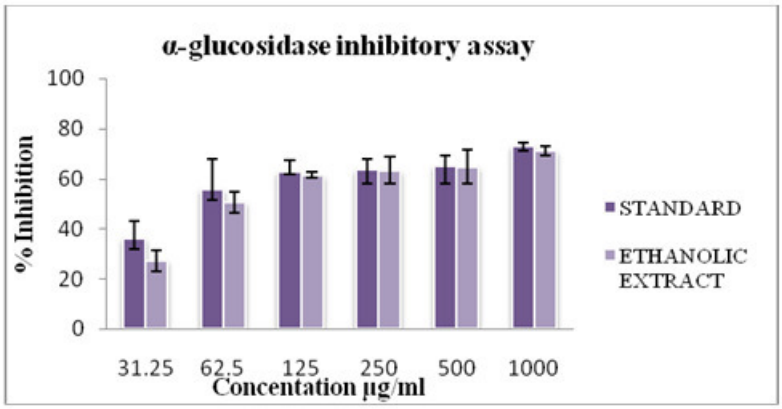
Table 6

Results of α - glucosidase inhibitory activity of Extract of karuppu Kavuni arisi

CONCENTRATION (µg/ml)	STANDARD (Mean±SD)	ETHANOL EXTRACT (Mean±SD)
31.25	36.11±6.91	27.26±4.17
62.5	55.56±12.4	50.73±4.18
125	62.82±4.44	61.45±1.22
250	63.55±4.28	63.45±5.37
500	65.06±4.32	64.86±6.83
1000	73.10±1.48	71.20±1.98

Graph 5

Representations of percentage inhibition of Extract of karuppu Kavuni arisi



α-Amylase inhibitory activity

The compounds that specifically act as amylase inhibitors are differently known as Starch Blockers, because they prevent dietary starch from being used by the body and in this way it lowers blood glucose level. The percentage inhibition of amylase by Kavuni extract was found to be 80.37%, on the other side the standard drug (Acarbose) shows 83.9% inhibition of amylase; in a way this proves that the karuppu Kavuni is a strong and effective anti-diabetic. The results of alpha amylase inhibitory assay are shown in Table 7and Graph 6.

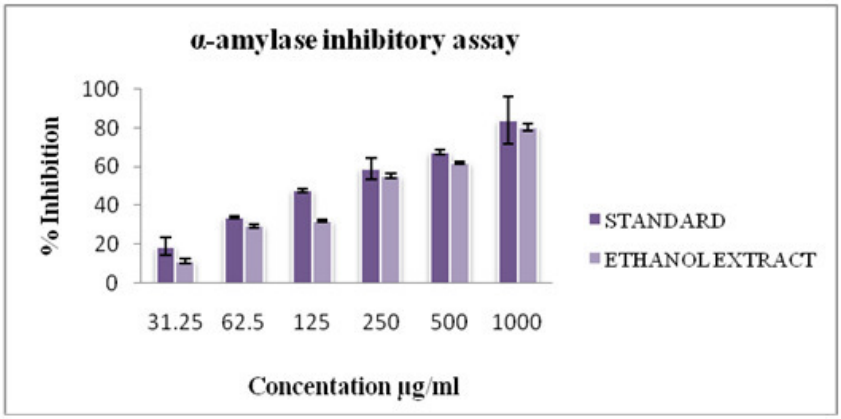
Table 7

Results of α - amylase inhibitory activity of Extract of karuppu Kavuni arisi

CONCENTRATION (µg/ml)	STANDARD (Mean±SD)	ETHANOL EXTRACT (Mean±SD)
31.25	19.08±4.40	11.7±1.21
62.5	34.04±0.42	29.5±1.07
125	47.81±1.07	32.13±0.57
250	59.15±5.46	55.25±1.28
500	67.8±1.20	62.09±0.43
1000	83.9±12.2	80.37±1.63

Graph 6

Representations of percentage inhibition of Extract of karuppu Kavuni arisi



CONCLUSION

The *in-vitro* studies carried out shows that Karuppu Kavuni arisi has more medicinal values. It has 5H-1,2,4-Triazolo[3,4-a][2]benzazepine,7-methyl-5-spirocyclohexane-(67%),Furan-2-carbohydrazide,N2-(3-indolylmethylene) (27.6%) present in abundant along with Alkaloids, Anthocyanin, Flavonoids, Anthraquinones, Coumarins, Phenols, Proteins, Carbohydrates, Tannins, Amino acids, Steroids, Glycosides, Terpenoids, and Saponins. From the review we know that it havs reduced levels of total soluble sugar, low fat content, increased protein content. From this we know thatithelps in the management of Diabetes Mellitus and reduce oxidative stress. It also, prevents protein denaturation up to an extent thereby reducing arthritis. In future, these constituents seen in kavuni rice can be isolated and tested for more medicinal properties. *In-vivo* studies can be conducted to prove that the intake of Kavuni grains can improve health by reducing diabetics and prevent arthritis occurrences. By this way the metabolism and bioavailability of the rice and its effect on organs can be established and evaluated.

CONFLICT OF INTEREST

Conflict of interest declared none.

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