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IN VITRO ANTIOXIDANT, ANTIBACTERIAL, ANTIDIABETIC AND ANTICANCER PROPERTY OF ORGANICALLY GROWN ARROWROOT POWDER

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ABSTRACT

Organic food consumption has been associated with reduced prevalence and complications of non-communicable diseases. There are limited studies on the therapeutic benefits of organically grown arrowroot in Kerala which can be the powerhouse of therapeutic benefits. Arrowroot (*Maranta arundinacea. L*) is a carbohydrate-rich plant with potential therapeutic properties. This study aimed to assess the antioxidant, anti-diabetic, antibacterial, and anti-cancer potential of organically grown arrowroot powder by subjecting it to GC-MS analysis, DPPH assay, FRAP assay, agar well diffusion method, alpha amylase inhibition assay and MTT assay. The presence of bioactive compounds in arrowroot powder were identified through GC-MS analysis. Quantitative analysis revealed the presence of phenols and flavonoids. DPPH radical scavenging activity and FRAP assay conducted on the sample showed a concentration-dependent increase, with a maximum activity of 33.16±3.618 percent at 150 µg/mL of arrowroot powder in DPPH assay and 81.86±1.45 percent at 30 µg/mL for FRAP assay. *In vitro* antibacterial activity demonstrated limited effectiveness against *Escherichia coli* at different concentrations of the sample. *In vitro* antidiabetic activity revealed maximum inhibition of alpha-amylase (37.21±0.56 percent) at 150 µg/mL of arrowroot powder. Alpha amylase inhibition activity increased with increasing concentration of arrowroot powder, indicating its potential anti-diabetic property. *In vitro* cytotoxicity by MTT assay

exhibited concentration-dependent effects, with a maximum inhibition of 39.55±1.77 percent observed at 100 µg/mL against lung cancer cells. The presence of bioactive compounds and the demonstrated therapeutic activities highlight the potential of organically grown arrowroot powder as a functional food ingredient with health benefits.

Keywords: Non communicable diseases, antioxidant, antibacterial, antidiabetic, cytotoxicity, therapeutic

INTRODUCTION:

Over the past few decades, the globe has witnessed a paradigm shift in mortality from communicable (infectious) diseases to non-communicable diseases (NCDs) **[1]**. The main causes of NCDs are poor dietary management, unchecked obesity, sedentary lifestyle, and a lack of understanding about maintaining good nutrition and health. NCDs, which include chronic lung disease, diabetes, cancer, and heart disease is estimated to blame for 74% of all deaths worldwide **[2]**. A method of farming known as "organic farming" produces healthy crops without the use of artificial agricultural inputs. There is no use of herbicides or chemical fertilisers, and crop rotation is frequently more diversified **[3]**. Diet can be modified by consuming organically grown foods which can prevent NCDs.

One such crop is arrowroot, which offers a variety of medicinal advantages including anti-inflammatory, hepatoprotective, anti-diabetic, antioxidant, and antibacterial properties **[4]**. In addition to being used as a culinary thickener, arrowroot flour has been used medically to treat irritable bowel syndrome-related diarrhoea **[4]**. There is potential for the creation of higher value-added products, particularly those that take advantage of the unique characteristics of plant starch and its therapeutic benefits. A review article explains the therapeutic benefits of arrowroot powder like antidiarrheal, antiulcer, antioxidant, antimicrobial, vibriocidal, immunostimulatory effects **[5]**. Organically grown foods have higher polyphenols and antioxidant content **[6]**. There are limited studies on the therapeutic benefits of organically grown arrowroot.

The aim of the study is to assess the *in vitro* antioxidant, antibacterial, antidiabetic and anti-cancerous properties of organically grown arrowroot powder. It is crucial for establishing a scientific understanding of its potential health benefits, identifying promising avenues for further research, elucidating underlying mechanisms, comparing its effects with existing treatments, and exploring potential therapeutic applications. Such studies contribute to advancing our knowledge in the field of natural compounds and their

potential role in promoting health and preventing diseases.

MATERIAL AND METHODS:

Sample Collection

Organically grown white arrowroot powder was collected from Bhoovara Agro Traders Pvt Ltd, Aluva, Ernakulam District, Kerala, India. Organically grown arrowroot was sourced from the agricultural lands of Manjali, Kerala.

Sample Preparation for *in vitro* experiments

The extract was prepared by dissolving the sample with ethanol solution in 1:1 ratio and left for cold extraction for 72 hours with constant shaking. The extract was then filtered using Whatman filter paper.

Estimation of *in vitro* antioxidant property and bioactive compounds of arrowroot (*Maranta arundinacea L.*) powder.

a) **Estimation of bioactive compounds using Gas Chromatography- Mass spectrometry technique.**

The GC-MS of the sample extract was performed in Shimadzu GC-MS to identify the bioactive compounds. The sample extract was injected into ELITE-5MS capillary column with 30 m x 0.25 mm ID and 0.25 µm thickness. The chromatographic conditions includes helium, carrier gas with a flow rate of 1 mL/min with a 80 °C column oven temperature and 260 °C injection temperature. The ion source temperature and interface temperature was kept at 230 °C and 280 °C.

b) **Estimation of phenols and flavonoids using Folin-Ciocalteau and Aluminium Chloride method.**

Phenols and flavonoids are the major classes of phytochemicals in plants **[7]**. The Folin-Ciocalteu method was used to estimate the phenolic content of arrowroot powder **[8]**. The working standard solution was pipetted into test tubes in amounts of 0.1, 0.2, 0.3, 0.4, and 0.5 ml. A volume of 0.125 ml of sample extract was added followed by distilled water and 0.125 ml of Folin's reagent. The contents in test tubes were incubated for 6 minutes followed by the addition of 1.25ml of 7 percent sodium carbonate. The absorbance was measured using spectrophotometer at 760 nm.

The total flavonoid content of arrowroot powder was determined using the Aluminium Chloride method **[9]**. Standard solution was pipeted into test tubes with concentrations of 0.5,1.0,1.5,2.0,2.0,2.5 ml. A concentration of 0.1 ml of sample was added into test tubes followed by distilled water, making upto a volume of 2.5 ml. In all the test tubes, 75 µl of 5 percent $NaNO_2$ solution was added and incubated at room temperature for 5 minutes. A volume of 150 µl of 10% Aluminium Chloride was incubated at room temperature for six

minutes followed by the addition of 0.5 ml of 1 M NaOH. This was mixed well and pink coloured substance was measured spectrophotometrically at 415 nm. The concentration of the sample was calculated from the standard curve of the graph by plotting the concentration of rutin along X axis and optical density along Y axis.

c) Determination of *in vitro* antioxidant property of arrowroot powder using DPPH and FRAP assay.

The antioxidant property of arrowroot powder was evaluated using the DPPH (alpha- diphenyl beta-picrylhydrazyl) free radical scavenging assay **[10]**. DPPH is a free radical which is converted to DPPH-H by hydrogen atom transfer, in the presence of an antioxidant. Various concentrations of the sample (50, 100 and 150 µg/mL) was added with 0.1 mM of DPPH solution along with 1 ml of ethanol. Each concentration of the sample were taken in triplicates and it was incubated at room temperature for 30 minutes along with control which did not contain the sample. Spectrophotometer was used to measure the purple colour's deterioration at 518 nm after 30 minutes of incubation. Radical scavenging activity per cent was calculated using the absorbance values (OD) of control and sample as follows:

$$\textbf{Radical scavenging activity percent} = \frac{\textbf{Control OD} - \textbf{Sample OD}}{\textbf{Control OD}} \times \mathbf{100}$$

In FRAP assay, ferric-tripyridyltriazine complex forms ferrous-tripyridyltriazine in the presence of antioxidants **[11]**. The FRAP reagent contained 2.5 mmol/L TPTZ, 20 mmol/L FeCl3, and 0.3 mol/L acetate buffer. The concentrations of the sample used were 30, 40 and 50 µg/mL. The absorbance was read at 593 nm and the reference solution was 1 mmol/L FeSO4. Ferric reducing antioxidant power per cent was calculated using the following equation.

$$\textbf{Ferric reducing antioxidant power per cent} = \frac{\textbf{Sample OD} - \textbf{Contol OD}}{\textbf{Sample OD}} \times \mathbf{100}$$

Determination of *in vitro* antibacterial property of arrowroot *(Maranta arundinacea L.)* powder.

Arrowroot powder was evaluated for antibacterial property against *Escherichia coli* by Kirby-Bauer's method.

a) **Inoculum preparation:** A loop of bacterial culture was introduced to 5 ml of SCDM (soyabean casein digest medium) and this was incubated at 30 ° C until it reached turbidity.

b) **Kirby-Bauer's method**: The Kirby-Bauer's method is a widely used technique to evaluate the antibacterial properties of powdered arrowroot **[12]**. Petri plates were sterilized by covering them with 25 ml of Mueller-Hinton agar material and letting them dry for 5 minutes. After that, cultures were swabbed, injected, and fashioned into wells using an 8 mm sterile cork borer. 100 ml of arrowroot powder was added using a micropipette, and the plates were then incubated for 24 hours at 37 °C. After incubation, the diameter of the zone of inhibition was measured with a ruler.

Estimation of *in vitro* antidiabetic property of arrowroot (*Maranta arundinacea L.*) powder.

Alpha amylase inhibition assay was used to assess antidiabetic properties of arrowroot powder, which can decrease postprandial synthesis of glucose, which may be therapeutic for diabetics **[13]**. The absorbance of the sample was measured at 540 nm and was compared to the blank solution. Different extract concentrations of 50, 100, and 150 ml in triplicates were placed in different test tubes with phosphate buffer with a pH of 6.9 added to the volume. The control was made by adding 0.5ml of phosphate buffer, 0.5ml of alpha amylase, 10 minutes of incubation at 25° C, 0.5ml of 1% starch solution, 1.0ml of DNS, 8ml of distilled water, and phosphate buffer (1ml) to measure the blank. The alpha amylase inhibitory activity was calculated using the following equation.

$$\textbf{Alpha amylase inhibitory activity percent} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Estimation of *in vitro* anticancerous property of arrowroot (*Maranta arundinacea L.*) powder.

a) **Cell lines and maintenance:** A549 Human lung cancer cells were purchased from National Centre for Cell Sciences (NCCS), located in Pune, India.

b) **Procedure:** MTT [3-(4,5-Dimethylthiazol-2yl)-2,5-biphenyl Tetrazolium Bromide] Assay was performed to evaluate anti cancerous property of arrowroot powder **[14]**. For this procedure initially, cells (2500 cells/well) were seeded on 96-well plates and given 24 hours to adjust to 37 °C and 5% CO2 culture

conditions. Test samples were made in DMEM media and added to the wells at final concentrations of 6.25, 12.5, 25, 50, and 100 g/mL. Triplicates of each experiment were performed and average results were used. Formazan crystals were formed and 100 L of 100% DMSO was added. A microplate reader was used to measure absorbance at 570 nm. Triplicates of each experiment were performed. The cell viability was expressed using the following formula:

$$\textbf{Percentage of cell viability} = \frac{(\textbf{Average absorbance of treated})}{\textbf{Average absorbance of control}} \times \textbf{100}$$

RESULTS

Estimation of *in vitro* antioxidant property and bioactive compounds of arrowroot (*Maranta arundinacea L.*) powder.

GC-MS analysis of arrowroot powder revealed compounds with pharmacological activities like antioxidant, antibacterial and anticancerous properties. The eluted compounds are shown in **Figure 1** and the bioactive compounds with pharmacological activities are shown in **Table 1**.

Table 1 shows the list of bioactive compounds found in arrowroot powder. Seven bioactive compounds were identified in arrowroot powder with antioxidant, antibacterial, and anticancerous properties.

Estimation of total phenolics and flavonoids

The phytochemical examination of arrowroot (*Maranta arundinacea L.*) powder revealed the presence of flavonoids and phenols. The phenols and flavonoids estimated in arrowroot powder was 1.62 µg/100mg and 49.2 µg/100mg.

Determination of *in vitro* antioxidant property of arrowroot powder using DPPH assay.

Arrowroot powder scavenges free radicals and improves scavenging activity with extract concentration. The maximum DPPH radical scavenging activity was 33.16±3.618 at 150 µg/mL concentration. Percent radical scavenging activity of arrowroot powder at different concentration using DPPH assay is given in **Table 2**.

One Way ANOVA followed by Tukey HSD showed that there was statistical significance difference in per cent radical scavenging activity between different concentration of sample ($p<0.05$). This shows that arrowroot powder has a good antioxidant capacity against free radical formation.

Determination of *in vitro* antioxidant property of Arrowroot powder using FRAP assay.

The results of the study showed that ferric reduction capacity of arrowroot powder increased with concentration. The highest Fe3+ reduction percent was 81.86±1.45 at a concentration of 30 µg/mL. Ferric reduction capacity of arrowroot (*Maranta arundinacea Linn.*) powder at different concentrations is shown in **Table 3**.

One Way ANOVA followed by Tukey HSD showed that there was statistical significant difference in reduction percent between different concentration of sample ($p<0.05$). This shows that the antioxidant power increased with concentration of arrowroot powder.

Determination of *in vitro* antibacterial property of Arrowroot (*Maranta arundinacea L.*) powder.

Antibacterial property is assessed quantitatively by measuring the diameter of the clear zone in the agar surface. **Table 4** shows that the bacterial effectiveness of arrowroot powder is low against organisms like *E. coli.*

Figure 2 shows the assessment of antibacterial activity in duplicates. **Figure 2** provides the evidence for poor antibacterial activity of arrowroot powder against *E-coli.*

Estimation of anti-diabetic property of arrowroot *(Maranta arundinacea L.)* powder.

Table 5 compares the antidiabetic property at different concentrations of arrowroot powder.

Arrowroot powder shows minimum inhibition of 15.28±0.84 percent at 50 µg/mL and maximum inhibition of 37.21±0.56 percent at concentration 150 µg/ml. It can be concluded that alpha amylase inhibitory activity of sample increased with increasing concentration of the sample, which means arrowroot *(Maranta arundinacea Linn.)* powder has anti-diabetic property. One way ANOVA followed by Tukey HSD showed that there was statistically significant difference between the percentage inhibition of alpha amylase activity at different concentrations of sample ($p<0.05$). Alpha amylase inhibitory activity of sample increased with increasing concentration of the sample, which means arrowroot (*Maranta arundinacea Linn*.) powder has anti-diabetic property.

Phase 4.

Estimation of anti-cancerous property of arrowroot powder *(Maranta arundinacea L.)*.

The per cent inhibition of arrowroot (*Maranta arundinacea L.*) powder at different concentrations using MTT assay was shown in **Table 6**.

Arrowroot powder shows a dose dependant reduction in cell viability of A549 human lung cancer cells, when administered with different concentrations of the sample. The maximum cytotoxicity was observed with 100 µg/ml of the sample. One Way ANOVA followed by Tukey HSD showed that there was a statistical significance difference in cell growth inhibition between different concentration of sample ($p<0.05$).

Figure 3A to 3F shows the microscopic images of cytotoxic effect of arrowroot on the cells at various concentrations of arrowroot powder.

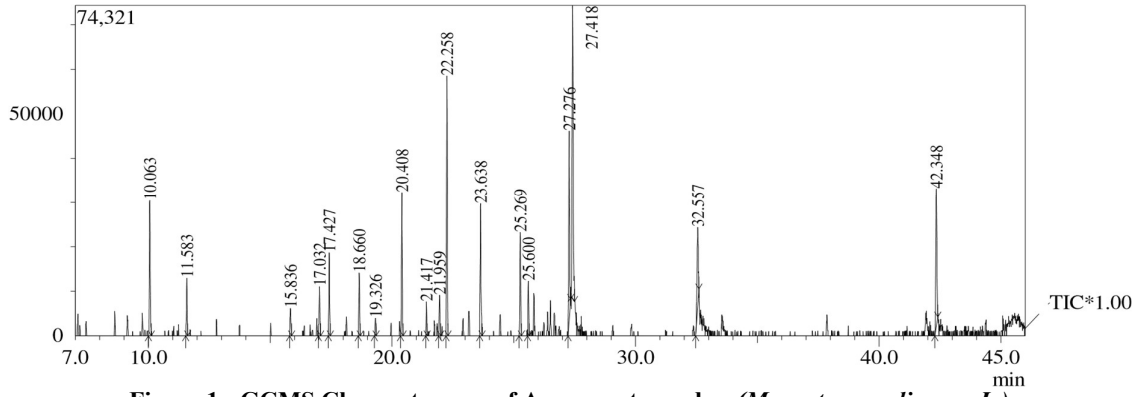

Figure 1: GCMS Chromatogram of Arrowroot powder. ***(Maranta arundinacea L.)***

Table 1: Bioactive compounds eluted and identified in arrowroot powder by GCMS Analysis

S. No.	RT	Compound name	Molecular weight(g/mol)	Molecular formula	Pharmacological Activity
1.	42.348	1,2-benzenedicarboxylic acid	166.13	$C_8H_6O_4$	1.2-Benzenedicarboxylic acid has antioxidant activity [15], anti bacterial effect against *Salmonella typhi, Streptococcus faecalis, Staphylococcus aureus* [16].
2	27.418	Hexadecanoic acid	256.42	$C_{16}H_{32}0_2$	Hexadecanoic acid has been reported to possess antioxidant, antimicrobial and anti-inflammatory activites [17].
3	25.269	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	278.34	$C_{16}H_{22}O_4$	The compound has antimicrobial activity against gram positive and gram negative bacteria as well as unicellular and filamentous fungi. It also possesses antioxidant and anticancer properties [18].
4	19.32	Methyl 2-[(aminosulfonyl) methyl]benzoate	215.23	$C_9H_9NO_4S$	It has a role as an xenobiotic metabolite [19].
5	17.427	Phenol, 3,5-bis(1,1-dimethylethyl)-	206.32	$C_{14}H_{22}O$	Phenol, 3,5-bis(1,1-dimethylethyl)- has antioxidant and some anti cancerous property [20].
6	18.660	Decanoic acid	172.26	$C_{10}H_{20}O_2$	Decanoic acid has antibacterial, anti inflammatory function [21].
7	10.063	Ethanol, 2-phenoxy-	164.20	$C_{10}H_{12}O_2$	Phenoxy ethanol has anti bacterial properties. It is effective against *Pseudomonas aeruginosa* [22].

Table 2: Percent radical scavenging activity of arrowroot (*Maranta arundinacea L.)* powder at different concentration using DPPH assay

Concentration (µg/mL)	Per cent radical scavenging activity in DPPH Assay
Control	0
50	10.27±0.891[a]
100	20.51 ±0.653[b]
150	33.16±3.618[c]

[a-c] Mean values with different subscript within a column shows that statistically significant difference ($p<0.05$)

Table 3: Ferric (Fe3+) reducing power activity of arrowroot powder

Concentration (µg/mL)	Percentage of Fe 3+ reduction
10	75.12 ± 2.91[a]
20	80.48±2.46[b]
30	81.86±1.45[c]

[a-c] Mean values with different subscript within a column shows statistically significant difference ($p<0.05$)

Table 4: Anti bacterial property of arrowroot powder (*Maranta arundinacea L.*)

SL. No.	Test organism	Zone of inhibition (mm)			Standard Drug Streptomycin (1000 ppm)	Test method
		100% (1:1in methanol)	50%	25%		
1.	*Escherichia coli (E.coli)*	No zone	No zone	No zone	21mm	Agar well diffusion method

Figure 2: Assessment of antibacterial activity in duplicates

Table 5: *Antidiabetic property of arrowroot (Maranta arundinacea L.) powder in different concentration*

Concentration(µg/ml)	Percentage inhibition
Control	0
50	15.28±0.84[a]
100	24.93333±0.44[b]
150	37.21867±0.56[c]

[a-c] Mean values with different subscripts within a column shows that statistically significant difference ($p<0.05$)

Table 6: Percent inhibition of Arrowroot *(Maranta arundinacea L.)* powder

Concentration of arrowroot powder (µg/ml)	Percent inhibition of sample
Control	0
6.25	6.5577±0.165[a]
12.5	15.523±1.778[b]
25	21.6±0.190[c]
50	29.836±2.656[d]
100	39.55±1.776[e]

[a-e] Mean values with different subscript within a column shows that statistically significant difference ($p<0.05$).

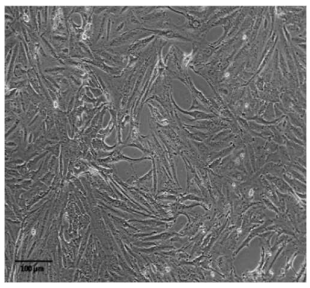

Figure 3A: Control

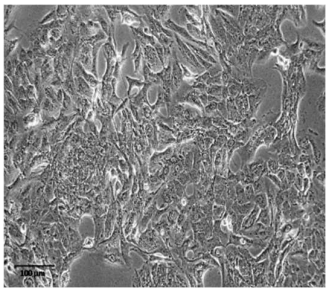

Figure 3B:6.25 µg/ml

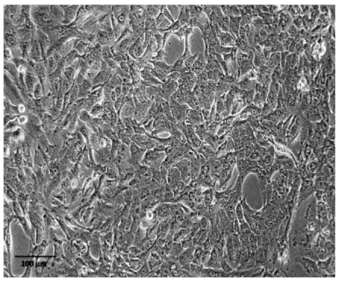

Figure 3C: 12.5 µg/ml

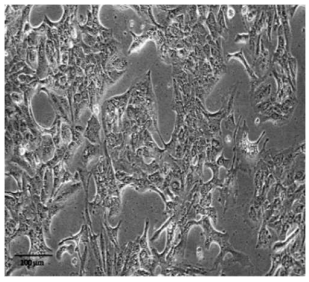

Figure 3D: 25 µg/ml

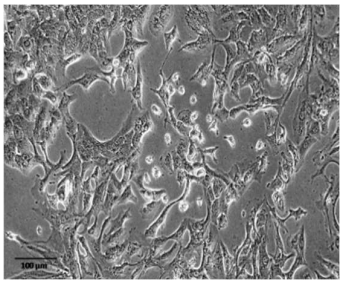

Figure 3E:50 µg/ml

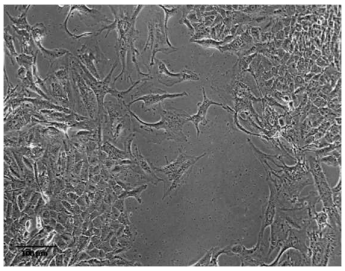

Figure 3F: 100 µg/ml

CONCLUSION:

The findings of the study showed that phenols and flavonoids in arrowroot powder have strong antioxidant activities to lessen the damaging effects of free radicals. Arrowroot powder has a variety of health benefits, such as antimicrobial, antibacterial, antidiabetic and antioxidant effects. MTT Assay showed strong anticancer activities against lung cancer cells, while its antibacterial properties had no impact on *Escherichia coli.* Further research is needed to determine the mechanism of action of the bioactive compounds present in the powder. The result of present research work is important to use arrowroot as a neutraceutical and to promote their use in prevention of risk of non communicable diseases. An adaptable plan should be established to maintain and encourage the use of organically cultivated crops to tap the pharmaceutical benefits out of it.

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