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**Research Article**

# Development and Characterization of Nutraceutical Cookies using Millet and Medicinal Leaves for Diabetic Health

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## ABSTRACT

Cookies are a crispy and delicious snack enjoyed by people of all ages, from young children to adults. However, most cookies are made using refined flour and sugar, which reduces the number of people who can consume them regularly. Therefore, to make a healthier alternative using easily available and nutritious ingredients, this Phyto cookie has been prepared by gluten free millet flour, Moringa oleifere, Mentha, and curry leaf. Moringa oleifera leaves notably used in medicinal purpose mainly used to treat diabetes mellitus and various disease. This study is focused at determining the efficacy of substitution of millet flour with Moringa oleifera leaf powder, curry leaf powder, Mentha leaf powder along with its physical, nutritional, bioactive, antioxidant and antidiabetic properties of developed cookies. In growing and increasing popularity had less health enrichment so the commercialization of nutritional rich Phytocookies among paediatric to geriatric population enhance their health benefit. This study is a novel strategy for replacement of biscuit manufacturing, to develop healthy cookies for supplementation of essential nutrient for all age group. The different blends of millet flour with Moringa oleifera leaf powder, Mentha leaf powder, and curry leaf powder. The sensory evaluation of the blended cookie sample was performed by using a 9-point hedonic scale method. The resulted largest mean score of the cookie was evaluated further for its nutrient content. Moringa leaf, Curry leaf and Mentha in cookies or any other baked foods may boost nutritional level and can be served as a novel approach to development of healthy cookies.

## INTRODUCTION

Diabetes is one of the most prevalent communicable diseases worldwide, diabetes poses significant challenges to public health systems, individuals and communities [1]. The International Diabetes Federation reported that around 425 million people worldwide had diabetes in 2017, with the figure projected to rise to cover 629 million by 2045 [2]. Diabetes prevalence has been

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rising steadily, fuelled by factors such as aging population, sedentary lifestyle, urbanization and unhealthy dietary habits and characterised by elevated blood glucose levels due to an absolute or relative deficiency in insulin production or function. *Moringa oleifera* is widely utilized as both food and medicine throughout the world [3]. *Moringa* can be used as a healing ailment for the treatment of diabetes, cancer, hypertension, malaria and fever [4]. Curry leaves (*Murraya koenigii*) activate the production of insulin in the body and thus possess hypo-glycemic properties, which help in regulating blood sugar levels [5]. Mentha leaves or mint leaves, are commonly used to add a refreshing, aromatic flavour to cookies providing a unique and pleasant taste [6]. Millets have great economic, health importance gluten free, have low glycaemic index and are known as "Nutra-cereals". Millets are rich source of carbohydrates, protein, crude fibre, phytochemicals, vitamins and minerals. There is a need to produce a value-added product from millets which improve food security and prevent micronutrient deficiency.

Cookies are the most popular bakery food consumed worldwide. A development of nutritional and diabetic friendly cookies production may help increase the conception of cookies with health conscious. Regular cookies often contain refined sugars which can lead to a spike in blood sugar levels and contribute to poor glycaemic control. As of recent data, approximately 30-40% diabetic patients might consume but the exact percentage can vary nutritional preference and the availability of diabetic friendly leaves, *Moringa oleifera*, Curry leaves (*Murraya koenigii*) and mint leaves (*Mentha*), incorporated millet cookies formulated, which enhances the conception and energises the diabetic population. In this research, the primary target to incorporate *Moringa* leaves and other innovative ingredients, such as Curry and *Mentha* leaf powders incorporated with millet flour, develop a unique and health-oriented cookie product that addresses both flavour and nutritional needs. The aim of this research, using manufacturing our nutritional rich Phyto cookies as a food supplement for Diabetic individuals gained more importance. The contribution of ingredients applied to produce high-quality end products with higher repeatability and accuracy. The goal is to create novel Phyto cookies that not only offer a refreshing and distinctive taste which provide substantial health benefits.

## MATERIALS AND METHODS

### COLLECTION OF THE HERBAL SAMPLES

The leaf samples were collected from farmland in Tirupur district (11.0374 N, 77.3540 E).

### EXTRACTION OF THE SAMPLES

The leaf samples were dried, and it was grinded into fine powder. The extraction was done by using a magnetic stirrer method in aqueous solution.

### QUANTITATIVE ANALYSIS OF PHYTOCHEMICAL SCREENING

The phytochemical analysis tests were carried out for the leaf samples. The colour changes were observed, like tannins, alkaloids, saponins, steroids, terpenoids, glycoside, phenol, alkaloids iodine, quinones, protein, carbohydrates and flavonoids [7].

### UV AND FTIR SPECTRUM ANALYSIS

The extract of *Moringa, Murraya,* and *Mentha* were analysed using a UV- visible spectrophotometer [8] and FTIR spectrum analysis [9]

## COLLECTION OF INGREDIENTS FOR COOKIES PREPARATION (INGREDIENTS)

The selected leaves *Moringa, Murraya,* and *Mentha* were washed and dried at ambient temperature for 2 weeks. The dried leaves were grained using an electric blender to obtain the leaf powders. Finger millet was washed and air dried, after the finger millet was milled using the electric blender and sieved to obtain the millet flour. Other ingredients like Jaggery, Butter, Salt were purchased from the local supermarket in Tirupur.

## PREPARATION OF PHYTOCOOKIES

The cookies are prepared using Millet lour, *Moringa, Murraya*, and *Mentha* leaf powder using the modified procedure described by AACC method 10-50.50. the cookies formulation is shown in table 1. Jaggery and butter were blended for 5 minutes using a mixer then the millet flour was added and mixed for 2 minutes and then *Moringa, Murraya,* and *Mentha* leaf powder were added, and salt cardamom powder was added and mixed baked at 200℃ for 25 minutes.

After baking the cookies were left to cool room temperature for 4 hours.

**Table 1. Ingredients for the Phyto cookies preparation**

| INGREDIENTS | QUANTITY IN GRAM AND MILLIGRAM |
|---|---|
| Finger millet flour | 150 |
| *Moringa* leaf powder | 6 |
| Curry leaf powder | 4.5 |
| Mint leaf powder | 4.5 |
| Jaggery | 50 |
| Butter | 50 |
| Salt | 0.05 |

## DETERMINATION OF PHYSICAL PROPERTIES

The physical properties of developed phyto cookies are determined (Diameter, Thickness, Spread ratio) [10].

### Diameter of the developed Phyto-Cookies

The diameter of the developed phyto cookies were measured using Vernier Calliper. The cookie was then rotated 90° and measured again for its diameter in centimetres. The process was repeated for three replicates, and the average diameter along with its standard deviation was calculated.

### Thickness of the developed Phyto-Cookie

Backed cookies were allowed to cool for approximately 30 minutes, the thickness of the cookies was measured Vernier Calliper. The procedure was repeated for three replicates and the average thickness, along with its standard deviation was calculated and reported in centimetres.

### Spread ratio of the developed Phyto Cookies

The spread ratio was calculated by dividing the diameter of the developed phyto cookie by its thickness, providing an indication of its quality. The average and standard deviation from three replicates measurements were determined and reported.

## FUNTIONAL PROPERTIES OF THE DEVELOPED PHYTO COOKIES

## DETERMINATION OF BULK DENSITY OF DEVELOPED PHYTO COOKIES

Developed phyto cookies sample was transferred into a od graduated cylinder, and the cylinder was tapped repeatedly until the sample level remained constant. The bulk density was then determined by dividing the weight of the sample by its volume [6].

## DETERMINATION OF SWELLING POWER OF DEVELOPED PHYTO COOKIES

The swelling power was determined by the method [11]. Sample was suspended in distilled water and heated at 50 °C for 30 mins. After the sample was allowed to cool and centrifuged at 3000 RPM for 20 minutes. The swelling power was calculated.

## ANTIMICROBIAL ACTIVITY OF DEVELOPED PHYTO COOKIES

## ABST ANALYSIS OF DEVELOPED PHYTO COOKIES

The nutrient broth was prepared and the test culture *E.coli*, *Staphylococcus aureus*, *Pseudomonas, Aspergillus* and *Candida* was inoculated. The broth was kept at 37°C for 3-4 days. The antibacterial activity test was carried out for the sample by using the test culture *E. coli*, *Staphylococcus aureus, Pseudomonas, Aspergillus* and *Candida*. The nutrient agar plate was prepared, and the test organisms was swabbed on it. Then add the sample into the well of different concentration (25 µl, 50 µl, 75 µl,100 µl). Then placed in the incubator at 37℃ for 24-48 hours. The zone of inhibition was observed and recorded.

## NUTRITIONAL EVALUATION OF DEVELOPED PHYTO COOKIES

Nutritional evaluation of the developed phyto cookies were determined by several methods.

## DETERMINATION OF REDUCING SUGAR

The developed phyto cookies was weighed and mixed it with distilled water. The content was heated slightly at water bath to dissolve the sugars and then filtered to obtain a clear extract. The sample was diluted with distilled water in the ratio of 1:1,1:2 and 1:3 respectively. The sample was diluted with distilled water and add Benedict's reagent to test tube and kept in a water bath at 40°C for 5-10 minutes. Observe the colour changes, it indicates the presence of reducing sugar [12].

**Table 2. Determination of reducing sugars**

| OBSERRVATION | RESULT |
|---|---|
| Blue | No reducing sugars |
| Green | Very low amount |
| Yellow | Low amount |
| Orange | Moderate amount |

## DETERMINATION OF CARBOHYDRATES

To determine the carbohydrates in the developed Phyto cookies sample. Phyto cookies sample was mixed with distilled water and heated slightly to extract the sugars. Filter the solution to obtain a clear extract, the sample was diluted with distilled water in the ratio of 1:1, 1:2 and 1:3 respectively. Add 1ml of Fehling's A and 1ml of Fehling's B solution to each test tube, these tubes were incubated in a water bath for 5-10 minutes at 40°C. Observe if there is any development of red precipitate. Notably, the result is positive if there is a formation of reddish-brown precipitate while the result is negative if there is no indication of such damage [13].

## DETERMINATION OF PESTICIDE

To determine the pesticides in the developed phyto cookies samples were mixed with distilled water and the contents were heated in a water bath and filters to obtain a clear extract. The sample was diluted with distilled water in the ratio of 1:1, 1:2 and 1:3 respectively. To this add 3ml of Bromophenol blue solution to each test tube and keep it in water bath for 5-10 minutes at 40℃. Bromophenol blue typically changes from yellow in acidic condition to blue I alkaline conditions. A change in colour indicates the presence of certain pesticides. No colour changes indicate no significant Pesticide interference or a neutral pH [14].

## DETERMINATION OF FAT

To determine the fat by using Distillation flask method, modified procedure of (Abayomi et al., 2013). 10 ml of the cookie sample were added in a conical flask to this 40ml diethyl ether and 20ml of distilled water were added. The content was poured into a Distillation Flask and incubated 24 hrs [15].

## DETERMINATION OF SALT

To determine the salt content by using modified version of Mohr's titration method [16]. 3.5g of sample were dissolved in 50ml of distilled water.

Titration process: Using silver nitrate solution and potassium chromate for titration process. Titrate the sample solution with silver nitrate (AgCl) and the chromate ions indicates the presence of excess silver ions

## DETERMINATION OF PROTEIN

The protein was determined by Lowry *et.al* method. The ilavaines citrate buffer and casine were added and incubated. After incubation trichloroacetic acid added and incubate at room temperature then sample were added to each tube after that alkaline copper solution was added and incubated for 10-20 minutes [17]. To this folinciocalteau was added and kept in dark incubation. Finally, the OD value was observed at 540nm using a calorimeter.

## DETERMINATION OF FIBRE

The fibre content determined by (AOAC 978.10). moisture and fat free sample was taken boiled sulfuric acid was added after addition the solution boiled 30min, maintain a constant volume by adding water at frequent intervals. After that the mixture filtered by using a filter paper. Then the residue was removed until free from acid and then add sodium hydroxide. The above procedure for repeat the solution then final residue was heated at 600°C for 2-3hrs.

## DETERMINATION OF HEAVY METALS

The sample was taken and diluted with distilled water and add aquaregia. The mixed solution placed on the hot plate. After that conc. Nitric acid was added. The sample allowed it to cool.

The solution was filtered by using Whatman filter paper [18].

## ANTIOXIDENT ACTIVITY BY DPPH ASSAY

Antioxidant activity was done with aqueous extract of the diabetic cookie for calculation of radial scavenging % and IC50 by DPPH assay. DPPH solution and methanol solution was used and absorption measured at 560nm [19].

$$DPPH\ Scavenged(\%) = \frac{Absorbance\ of\ blank\ at\ 0\ min - Absorbance\ of\ the\ antioxident\ at\ 30min\ X\ 100}{Absorbance\ of\ blank\ at\ 0\ min}$$

## ANTIDIABETIC ACTIVITY

## AMYLASE INHIBITION ASSAY

A mixture of the sample and sodium phosphate buffer containing α-amylase solution was incubated at room temperature for 10 minutes. Subsequently 1% starch solution was prepared in the same buffer was added to the reaction mixture which was incubate at 25°C for 10 minutes. To terminate the reaction dinitro salicylic acid was added, and the mixture was placed in boiling water bath for 5 minutes before cooling [6]. The absorbance was measured at 540 nm, and the

percentage inhibition was calculated using the formula:

## GLUCOSIDASE INHIBITION ASSAY METHOD

The sample was mixed with α-glucosidase solution, prepared in phosphate buffer and preincubated. After that nitrophenyl-glucopyranoside solution prepared in the same buffer then absorbance was measured at 405nm [6].

$$Percentage\ inhibition\ of\ glucosidase = \frac{Absorbance\ of\ control - absorbance\ of sample\ X\ 100}{Absorbance\ of\ the\ control}$$

## SENSORY EVALUATION OF DEVELOPED COOKIES

The developed phyto cookies was subjected to a sensory evaluation using hedonic scale The Cookies were evaluated for colour, flavour, texture, taste and overall quality using nine-point hedonic scale, ranging from highly unpleasant to highly pleasant method [20].

## RESULTS AND DISCUSSION

## QUALITATIVE ANALYSIS OF PHYTOCHEMICAL ANALYSIS

The results of the phytochemical screening of the leaf sample extract. The results, the extract containing tannin, alkaloids, phenol saponins, proteins, flavonoids and carbohydrates showed positive result. Which means the *Moringa*, Curry leaf and Mint can be considered a rich source for phyto nutrients.

### *Moringa oleifera*

The phytochemical analysis of *Moringa oleifera* using aqueous extract containing tannin, alkaloids, saponins, phenol, protein, carbohydrate, flavonoids were positive results and the steroids, terpenoids, quinone and alkaloids iodine were negative results.

### *Murraya koenigii*

The phytochemical analysis of *Murraya koenigii* using aqueous extract, containing tannin, alkaloids, phenol, protein and carbohydrates were positive results and saponins steroids quinone, terpenoids glycosides flavonoid and iodine were given negative result

### *Mentha piperita*

The phytochemicals analysis of *Mentha* piperita using aqueous extract, containing tannin alkaloids phenol protein and carbohydrates were given positive results and saponins steroids terpenoids glycosides quinone flavonoid and iodine were negative results.

## UV SPECTROSCOPY

The extracts were analysed using a UV- visible spectrometry at 200-1100nm wavelength to examine their absorption spectrum. The peak values of the UV- visible spectrum were given below

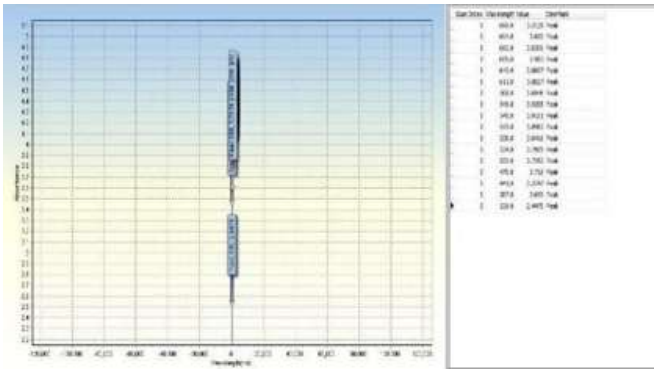

**Fig 1: UV spectroscopy of *Moringa oleifera***

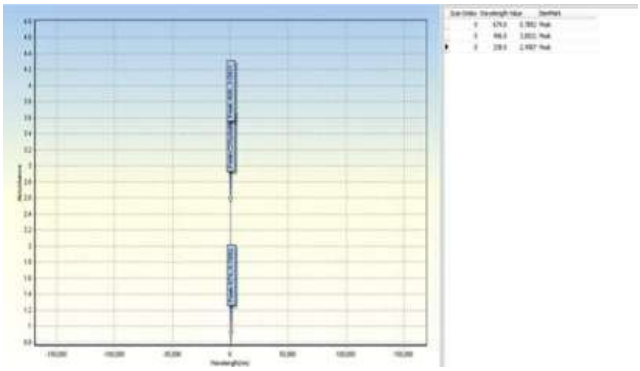

**Fig 2: UV spectroscopy of *Murraya Koenigii***

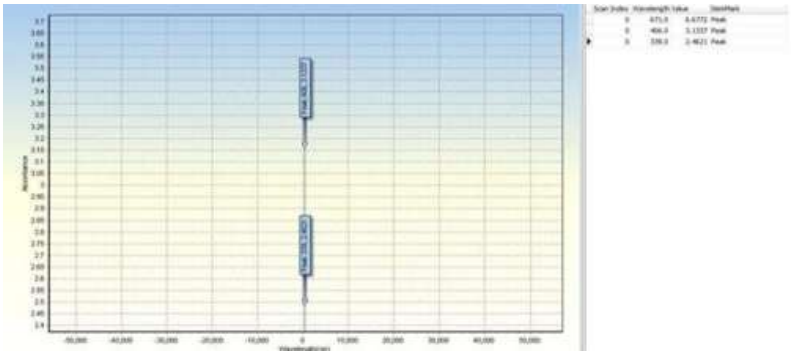

**Fig 3: UV spectroscopy of *Mentha***

## FTIR SPECTRUM ANALYSIS

In FTIR spectrum analysis of *Moringa oleifera, Mentha, Murraya koenigii* extracts revealed the presence of various functional groups including hydroxyl (-OH), carbonyl (C=O), alkenes (C=C), alkanes (C-H), ethers (C-O), and nitro compounds (N-O). These functional groups were identified by their characteristic peaks at 3289 $cm^{-1}$ (O-H/N-H stretching), 1630.52 $cm^{-1}$ (C=C or C=O stretching), 1541.81 $cm^{-1}$ (N-O asymmetric stretching), 1215.9 $cm^{-1}$ (C-O stretching), and 1051.01 $cm^{-1}$ (C-O stretching in carbohydrates or glycosides).

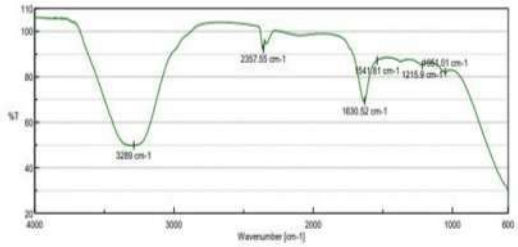


**Fig 4: FTIR spectrum result for *Moringa***

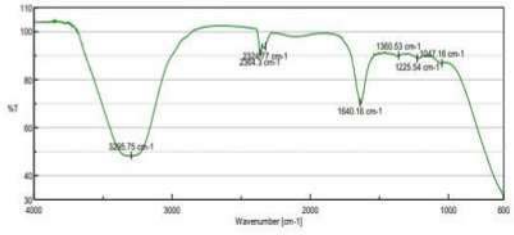


**Fig 5: FTIR spectrum result for *Murraya***

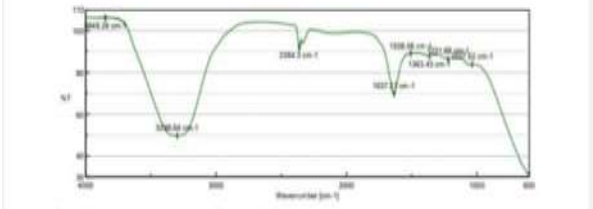

**Fig 6: FTIR spectrum result for *Mentha***

## PREPARATION OF PHYTO COOKIES

**Fig 7: Phyto cookie**

**Table 3: Result of bulk density and swelling power**

| Sr. No | FUNCTIONAL PROPERTIES | RESULT (g/ml) |
|---|---|---|
| 1. | Bulk Density | 0.54996g/ml |
| 2. | Swelling Power | 1.5ml/g |

## PHYSICAL PROPERTIES OF DEVELOPED PHYTO COOKIES

The Mean SD diameter of the phyto cookie 5.14+-0.071.

THE Mean SD thickness of the phyto cookie 0.91+-0.02.

The Mean SD spread ratio of the phyto cookie 5.62+-0.15.

**Table 4: Result of diameter, thickness and spread ratio**

| SR. NO | PHYSICAL PROPERTIES | SAMPLE 1 | SAMPLE 2 | SAMPLE 3 | MEAN ± SD |
|---|---|---|---|---|---|
| 1. | Diameter (cm) | 5.22 | 5.13 | 5.08 | 5.14 ± 0.071 |
| 2. | Thickness (cm) | 0.89 | 0.90 | 0.93 | 0.91 ± 0.021 |
| 3. | Spread ratio | 5.8 | 5.7 | 5.5 | 5.6 ± 0.15 |

## FUNCTIONAL PROPERTIES OF DEVELOPED PHYTO COOKIES

The bulk density of Millet flour cookies with *Moringa*, Mint and curry Leaf powder was found to be 0.54996 g/m. Swelling power was found to be 1.5 ml/g.

## ANTIMICROBIAL ACTIVITY OF DEVELOPED PHYTO COOKIES

The Antimicrobial assay helped to determine the resistance and sensitivity of organism to the *Moringa, Murraya, Mentha* leaf extract and Phyto cookies. *E.coil, S.aureus, Psudomonas,* and *Aspergillus* were sensitive to the *Moringa, Murraya, Mentha* and the developed phyto cookies, *Candida* was resistant to the *Moringa, Murraya*, *Mentha* and the phyto cookies.

**Table 5: ABST result of *Moringa***

| ORGANISM | 25 ML OF EXTRACT | 50 ML OF EXTRACT | 75 ML OF EXTRACT | 100 ML OF EXTRACT |
|---|---|---|---|---|
| *Escherichia coli* | 17mm | 17mm | 18mm | 18mm |
| *Staphylococcus aureus* | 23mm | 25mm | 26mm | 27mm |
| *Pseudomonas* | 23mm | 24mm | 25mm | 26mm |
| *Aspergillus* | 15mm | 16mm | 17mm | 18mm |
| *Candida* | Nil | Nil | Nil | Nil |

**Table 6: ABST result of *Murraya***

| ORGANISM | 25 ML OF EXTRACT | 50 ML OF EXTRACT | 75 ML OF EXTRACT | 100 ML OF EXTRACT |
|---|---|---|---|---|
| *Escherichia coli* | 20mm | 20mm | 21mm | 21mm |
| *Staphylococcus aureus* | 23mm | 25mm | 26mm | 27mm |
| *Pseudomonas* | 23mm | 24mm | 25mm | 26mm |
| *Aspergillus* | 15mm | 16mm | 17mm | 18mm |
| *Candida* | Nil | Nil | Nil | Nil |

**Table 7: ABST result of *Mentha***

| ORGANISM | 25 ML OF EXTRACT | 50 ML OF EXTRACT | 75 ML OF EXTRACT | 100 ML OF EXTRACT |
|---|---|---|---|---|
| *Escherichia coli* | 20mm | 21mm | 23mm | 24mm |
| *Staphylococcus aureus* | 25mm | 25mm | 27mm | 28mm |
| *Pseudomonas* | 23mm | 24mm | 24mm | 26mm |
| *Aspergillus* | 19mm | 22mm | 24mm | 26mm |
| *Candida* | Nil | Nil | Nil | Nil |

**Table 8: Result for Nutritional properties of developed phyto cookies**

| Sr. No | NUTRITIONAL PROPERTIES | RESULTS |
|---|---|---|
| 1 | Reducing Sugars | Present |
| 2 | Carbohydrates | Present |
| 3 | Pesticides | Absent |
| 4 | Fat | 0.44g |
| 5 | Salt | 0.35g |
| 6 | Protein | 0.375mg |
| 7 | Fibre | 1.009g |

## NUTRITIONAL PROPERTIES OF DEVELOPED PHYTO COOKIES

The Nutritional properties of developed phyto cookies results indicated the presence of reducing sugar and carbohydrates. While pesticides were absent. The measured salt content was 0.04%, fibre content was 0.673%, fat content was 0.049%, Salt concentration was 0.35g and protein content was 0.375mg.

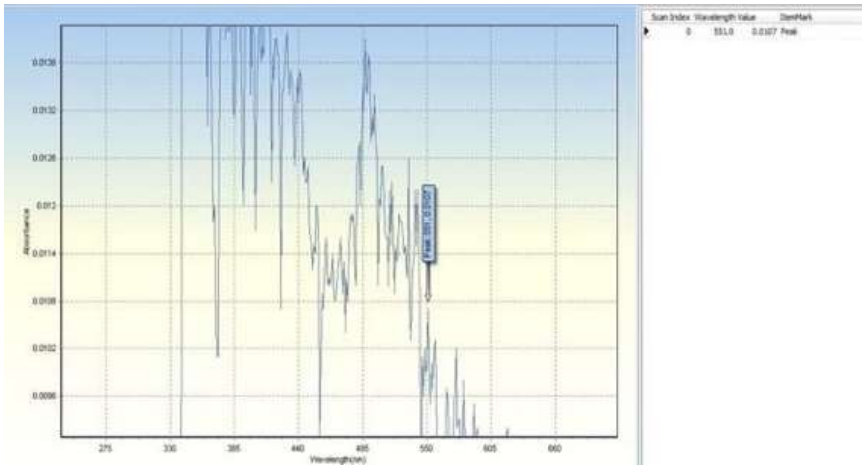

**Fig 8: Heavy metal analysis of developed phyto cookies**

## ANTIOXIDANTS ACTIVITY OF DEVELOPED PHYTO COOKIES

The Results of the antioxidant properties of developed phyto cookies Ic50 value is 2330.21

**Fig 9: Antioxidant activity of developed phyto cookies**

| Sr. No | CONCENTRATION | CONTROL ABSORBANCE AT (560nm) | SAMPLE ABSORBANCE AT (560nm) | RSA% | IC50 |
|---|---|---|---|---|---|
| 1. | 100 | 0.33 | 0.11 | 66.66 | 2330.21 |
| 2. | 200 | 0.33 | 0.07 | 78.78 | |
| 3. | 300 | 0.33 | 0.08 | 75.75 | |
| 4. | 400 | 0.33 | 0.08 | 75.75 | |
| 5. | 500 | 0.33 | 0.09 | 72.72 | |

## ANTIDIABETIC ACTIVITY OF DEVELOPED PHYTO COOKIES

The results indicate a significant increase in enzyme inhibition as the concentration of *Moringa, Murraya* and *Mentha* leaf powders increased in the cookies. Notably highest fortification level exhibited the lowest IC50 value. Inhibition of Alpha Amylase is 27.08+-16.86% and the IC50 value of Alpha Amylase is 471.59. Inhibition of Alpha Glucosidase is 20.00+-12.28% and IC50 value of Alpha Glucosidase is 2,173. 40.

**Table 10: Alpha amylase inhibition of developed phyto cookies**

| SR. NO | SAMPLE VOLUME | ABSORBANCE | % INHIBITION | % AMYLASE ACTIVITY | IC 50 |
|---|---|---|---|---|---|
| 1 | 100 | 0.48 | 0.00 | 100.00% | 471.59 |
| 2 | 200 | 0.37 | 22.92 | 77.08% | |
| 3 | 300 | 0.33 | 31.25 | 68.75% | |
| 4 | 400 | 0.29 | 39.58 | 60.42% | |
| 5 | 500 | 0.28 | 41.67 | 58.33% | |

**Table 11: Glucosidase inhibition assay of developed phyto cookies**

| SR. NO | SAMPLE VOLUME | ABSORBANCE | % INHIBITION | % GLUCOSIDACE INHIBITION | IC 50 |
|---|---|---|---|---|---|
| 1 | 100 | 0.25 | 03.84 | 96.16 | 2,173.40 |
| 2 | 200 | 0.23 | 11.54 | 88.46 | |
| 3 | 300 | 0.20 | 23.07 | 76.93 | |
| 4 | 400 | 0.19 | 26.92 | 73.08 | |
| 5 | 500 | 0.11 | 57.69 | 65.39 | |

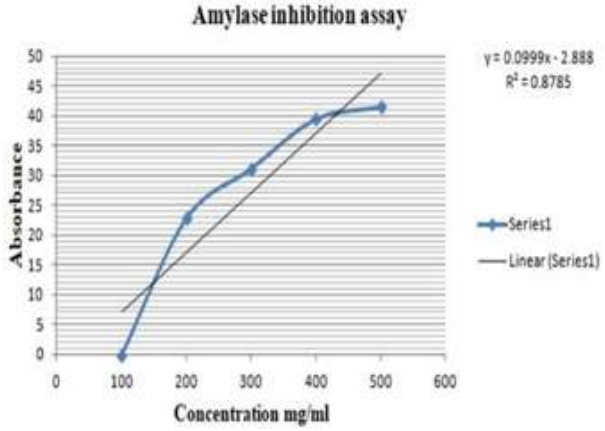


**Fig 10: Alpha amylase inhibition**

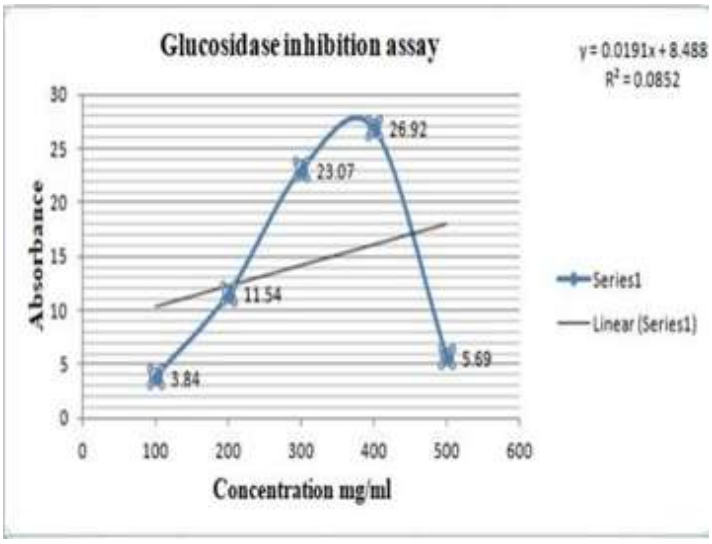


**Fig 11: Alpha glucosidas inhibition of developed phyto cookies of developed phyto cookies**

## SENSORY EVALUATION

**Table 12: Sensory evaluation of developed phyto cookies**

| CHARACTERISTICS | PHYTO COOKIES |
|---|---|
| Colour | 7.5 |
| Flavour | 8.5 |
| Taste | 9.5 |
| Texture | 8 |
| Overall Quality | 9 |

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