

Green Tea Formulation

Submitted in partial fulfillment of the requirements for the award
of Bachelor of Technology Degree in Biotechnology
by

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF**

BIO AND CHEMICAL ENGINEERING

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY (DEEMED TO BE
UNIVERSITY)**

Accredited with Grade “A” by NAAC JEPPIAAR

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**DEPARTMENT OF
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of M.A SINDHUJA(37230043) who carried out the project entitled "GREEN TEA FORMULATION" under our supervision from jan 2021 to March 2021

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Submitted for Viva voce Examination held on 15 april

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DECLARATION

I M.A SINDHUJA (3723043) hereby declare that the Project Report entitled "GREEN TEA FORMULATION" done by me under the guidance of dr V. RAMESH KUMAR , Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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PLACE: Bangalore

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ABSTRACT

Green tea is considered is the one of the healthy beverages on the planet. It contains many antioxidants and has many health benefits like protecting against cancer, lowering risk of heart disease and aiding in weight loss. Green tea contains many bioactive compounds and rich in polyphenols. A natural green tea formulation was made by using two natural fruit extracts orange and grape. Natural preservative like honey were used then followed by a contamination check and the pure sample is then subjected to Anti-microbial followed by phytochemical analysis (qualitative & quantitative) and antioxidant test is performed. As per the protocol the given green tea formulation is designed and observed for further studies.

Keywords: Green tea Base, orange & Grape extract, phytochemical analysis, Anti-oxidant, Anti-microbial test

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ABBREVIATION

- 1. EGCG** - EPIGALLOCATECHIN-3-GALLATE
- 2. AHR** - ARYL HYDROCARBON RECEPTOR
- 3. QAMS** - QUANTITATIVE ANALYSIS OF MULTI
COMPONENTS BY SINGLE MATERIAL
- 4. HPLC** - HIGH PERFORMANCE LIQUID CHROMOTOGRAPHY
- 5. FRAP ASSAY** - FERRIC REDUCING ABILITY OF PLASMA
- 6. FTC ASSAY** - FERRIC THIOCYANATE ASSAY-
- 7. MIC** - MINIMUM INHIBITORY CONCENTRATION
- 8. DPPH** - 1,1 DIPHENYL – 2 – PICRYL HYDRAZYL
- 9. NAOH** - SODIUM HYDROXIDE
- 10. HCL** - HYDROGEN CHLORIDE

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CHAPTER-1 INTRODUCTION:

1.1 GREEN TEA

green tea can be made in different types u can enjoy it both hot and cold and can still reap the goodness of tea. Both the leaves and the tea itself can also be incorporated into cooking is a type green tea of tea that is made from *Camellia sinensis* leaves and buds that have not undergone the same withering and used to make oolong tea and Black tea Green tea originated in china, but its production and manufacture has spread to other countries in East asia.

1.1.1HEALTH BENEFITS

Green tea is enjoyed in various form all round the globe .The green tea plant contains a range of healthy compounds that make it into the final drink Tea is rich in polyphenols, and other compounds that makes it a healthier option as it contains properties, such as reducing inflammation and aid help to fight cancer. Green tea has certain components like catechin called epigallocatechin-3-gallate (EGCG). Catechins are present in the green tea which are antioxidants that help prevent cell damage. Green tea is made from unoxidized leaves. Which can offer various health benefits.

1.1.2 CONSTITUENTS

It is rich in nutrient and antioxidants. The substances present in green tea can reduce the formation of free radicals in the body, protecting all the cells and molecules from vital damage. Those free radicals play a role in aging and many types of diseases. EGCG is considered as one of the most powerful compounds present in green tea. It appears to be one of the main compounds that gives green tea its medicinal properties. It contains small amounts of minerals to it and has many health benefits

1.1.3 RESEARCH CARRIED IN GREEN TEA

However research shows that 78 percent of the tea is Consumed worldwide out of which is green tea. Green tea was used as reserch states back it was also used in traditional Asian medicine to treat various diseases, helps in good digestion, can enhance heart and mental health, and regulate and control body temperatures. Many studies have shown green tea can show some positive results on everything from weight loss to liver disorders, type 2 diabetes, and Alzheimer's disease.

1.2 FRUIT EXTRACT

Fruits have many benefits on health and they are naturally sweet and it's has many minerals and nutrients and help for body growth. Fruits has many essential minerals and vitamins and has antioxidants which boosts the health and can reduce the heart disease and inflammation

1.2.1 BENEFITS OF ORANGE

Oranges are considered as one of the easy sources of vitamin c and they can help in boosting in immune system and it can prevent skin damage and it can keep skin stay healthy and prevents ageing and it can also lower cholesterol and controls the blood sugar levels. It can also help in lowering the risk of cancer as it has antioxidants and large amounts of vitamin c and they are good option as it has low calories which makes it ideal to for weight loss and help boost the immunity.

1.2.2 BENEFITS OF GRAPES

Grapes are packed with lots of minerals and, mainly vitamins c and vitamin k. Grapes has high amount of antioxidant contents and may prevent many health disease and it can boost the overall health and immunity and it can keep blood sugar in control and can fights against cancer and can fight diabetes we can enjoy grapes in different forms like juice, wine, frozen or fresh.

1.3 BIOPOTENTIAL ACTIVITY

1.3.1 PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical analysis was used to analyze the presence of compounds namely Alkaloids, Flavonoids, Saponins, Tannins, Phenols, Proteins, Cardiac glycosides, Terpenoids, Carbohydrate and Quinones. (Solomon Charles Ugochukwu et al., 2013)

1.3.2 ANTIMICROBIAL ACTIVITY

Antimicrobial activity is the process of killing or stopping the growth of different microorganism like bacteria, fungi and viruses. (Hemanta koley et al., 2019)

1.3.3 ANTIOXIDANT ACTIVITY

Antioxidant substances can slow or prevent damage to cells caused by free radicals present in our body. Probiotic can produce more metabolic compounds with antioxidant activity, such as glutathione, butyrate and folate.



Fig 1.1 GREEN TEA



**Fig 1.2.1 GRAPE EXTRACT +GREEN TEA
EXTRACT+GREEN**



Fig 1.2.2 ORANGE

CHAPTER -2

LITERATURE

SURVEY

Kris Cunnars et al., (2020), studied that Green tea is touted to be one of the healthiest beverages on the planet. It's loaded with antioxidants that have many health benefits, which may include. improved brain function, fat loss, protecting against cancer, lowering the risk of heart disease.

It Suko Fukuda et al., (2004) in In this study, we identified lutein and chlorophyll a and b from green tea leaves as the novel antagonists for AHR. These active compounds suppressed AHR transformation dose-dependently.

Saeed .M & Naveed. M et al., (2017), studied about The effects of green tea include anti-oxidative, anti-inflammatory, anti-arthritis, anti-stress, hypolipidaemic, hypocholesterolaemic, skin/collagen protective, hepatoprotective, anti-diabetic, anti-microbial, anti-infective, anti-parasitic, anti-cancerous, inhibition of tumorigenesis and angiogenesis, anti-mutagenic, and memory and bone health-improving activities. Apart from its utilization in humans, green tea has also played a significant role in livestock production such as in dairy, piggery, goatry and poultry industries.

Apples are a popular fruit, containing antioxidants, vitamins, dietary fiber, and a range of other nutrients. Due to their varied nutrient content, they may help prevent several health conditions. Apples come in a variety of shapes, colors, and flavors and provide a range of nutrients that can benefit many different aspects of a person's health Jillian Kubala et al., (2019)

Sushmita et al., (2016). The study tells that apples High in pectin fiber apples boost metabolic levels, help improve heart health and regulate body's blood sugar levels by regulating the release of sugar. They are also packed with vitamin and minerals that promote healthy bones, teeth and skin. Apples are loaded with vitamin C that boosts immunity, keeping any ailment at bay. They also consist of healthy polyphenol antioxidants that are essential for overall health. In fact, according to a study published in the Journal Planta Medica, animal studies have shown apples and apple juice to be effective in reducing the risk of colon, skin and breast cancers.

(Sara .v & peter .F et al., 2003) Accordingly, a new study was carried out to evaluate the protective impact of fresh apple skin extract against glycated protein-iron chelate induced toxicity in human umbilical vein endothelial) in vitro⁵.

Grape extract have been improving the anti-inflammation condition not only in gastrointestinal tract but also the entire body. There found anti-inflammatory activity of grape pulp. (Yuri Kato et al. 2015).

(Poongothai., 2015) In the present study, the phytochemical screening of the powder of leaf of Lantana Camara methanolic extract was executed. The results of qualitative phytochemical screening of methanolic extract of Lanatana camara leaves revealed that the presence of various bioactive secondary metabolites constituents in alkaloids, flavonoids, phenols, tannins, saponins, carbohydrates, amino acids, steroids, terpenoids and protein were respectively. The quantitative phytochemical analysis of methanolic extract of Lanatana camara leaves showed that the maximum concentration of flavonoids (43.31 ± 0.081) followed by Alkaloids (35.14 ± 0.029) Phenol (25.14 ± 0.094) and Tannins (15.05 ± 0.026) were respectively. Hence, Lanatana camara are used for discovering and analysis of the Secondary metabolites which are precise helpful for the manufacturing of new drugs for treatment of various diseases

Grapes are a powerhouse of antioxidants – they contain a wide range of phytonutrients right from carotenoids to polyphenols. Studies have revealed that these phytonutrients help in preventing certain kinds of cancers and help in maintaining heart health. Among polyphenols, resveratrol is known for its miraculous properties such as inhibiting the formation of free radicals that could cause cancer and dilating blood vessels to ease blood flow and low blood pressure. Point to note: The antioxidant content is the highest in the seeds and the skin. So, do make use of them. (Plavaneeta Borah et al., 2014)

Red grapes contain fewer calories than green grapes. They are used to make red wine, jams, jellies, grape juice, or consumed just raw. Red grapes contain Vitamins A, C, B6, potassium, calcium, iron, phosphorus, folate, magnesium and selenium. Flavonoids are the most powerful antioxidants found in grapes that contribute to ocular health (Jayashree Bhagat et al., (2013)) Saanvi et al., (2020) small portion of black grapes is more than enough in controlling the levels of sugar in the blood. Resveratrol in grapes is responsible for striking the right balance in the insulin levels as well as insulin sensitivity. The low levels of Glycemic index work in your favour here. By a good increase in the blood flow, it also helps in balancing the blood pressure levels.

Satyajit D.Sarker et al., (2011) studied The strategy combining quantitative analysis of multi-components by single marker (QAMS) with charged aerosol detection (CAD) has certain advantages for quantifying multiple components. However, relevant research was very limited. In this study, a high-performance liquid chromatography (HPLC)-CAD-QAMS method was successfully developed for the multi-component determination of Qishen Yiqi dripping pills. Factors that affect the performance of the HPLC-CAD-QAMS approach were sufficiently investigated. After the method verification, principal component analysis was applied to evaluate the quality consistency of three types of samples

(Sahira Banu., et al 2013) Plants are recognized in the pharmaceutical industry for their broad structural diversity as well as their wide range of pharmacological activities. The biologically active compounds present in plants are called phytochemicals. These phytochemicals are derived from various parts of plants such as leaves, flowers, seeds, barks, roots and pulps. These phytochemicals are used as sources of direct medicinal agents. They serve as a raw material base for elaboration of more complex semi-synthetic chemical compounds. This paper mainly deals with the collection of plants, the extraction of active compounds from the various parts of plants, qualitative and quantitative analysis of the phytochemicals.

Rituparna Chatterjee et al., (2010) Several factors influence the stability of foods and ingredients including added and natural antioxidants, temperature, handling conditions and exposure to light and oxygen. Properties associated with this decline in freshness include undesirable changes in flavors, textures, shelf stability, nutritional content and appearance. Many tests have been developed and used over the years to quantify the antioxidant and free-radical scavenging properties of foods, including the DPPH assay, FRAP assay, Fox assay and FTC assay. Each of these tests has its advantages and disadvantages depending on the sample type and the ease of performing the assay

Antimicrobial activity antibacterial agent, minimum inhibitory concentration (MIC) values for the most common Gram-positive and Gram-negative pathogens are tabulated with members of the same drug group appearing in the same Table. Since published MIC values differ, sometimes quite widely, depending on the methodology used and the source of the micro-organisms tested, those given are representative ones, usually based on fully susceptible strains. Activity against other relevant pathogens is described in the sample(A.J.Dart & C.M Dart et al.,(2017).

CHAPTER-3

AIM AND OBJECTIVE

3.1 AIM

1. To formulate a green Tea based health drink
2. To analyze the biopotential activity of the prepared formulation

3.2 OBJECTIVE

1. Formulation of the green tea
2. Adding two natural Fruit based concentrates
3. Analyzing the biopotential activity of the prepared formulation viz,
 - Phytochemical analysis
 - Antioxidant activity
 - Antimicrobial activity

CHAPTER-4

MATERIAL AND METHODS

4.1 MATERIAL REQUIRED

4.1.1 CHEMICALS REQUIRED

20% NaOH, 70% dilute HCl, methanol and ethanol extracts, Wagner's reagent, 10% of alcoholic ferric chloride, 1% copper sulphate, chloroform

4.1.2 INSTRUMENT REQUIRED

UV spectroscopy, autoclave, colorimeter, incubator, Test tubes, weighing balance, microscope and hot water bath.

4.2 METHODOLOGY

4.2.1 SAMPLE PREPARATION

- Green tea leaves (2gm) added to water (200ml) at 60 degree c and and brewed for around 5 min and cooled
- .Orange Extract: orange pulp is boiled and made into concentrate with constant stirring(4ml)
- Grape extract grape pulp is taken and boiled and made into thick concentrate (4ml)
- To the green tea these fruit concentrates are added along with natural preservatives like honey (3gm)

4.2.2PHYTOCHEMICAL ANALYSIS

4.2.3 QUALITATIVE PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical analysis was used to analyze the presence of compounds namely Alkaloids, Flavonoids, Saponins, Tannins, Phenols, Proteins, Cardiac glycosides, Terpenoids, Carbohydrate and Quinones. (Solomon Charles Ugochukwu et al., 2013)

1.Determination of alkaloids

To 2ml of methanol and ethanol extracts, few drops of Wagner's reagent were added. Formation of reddish brown precipitate indicates the presence of alkaloids.

2.Determination of flavonoids:

In 2 ml of each extract, few drops of 20% NaOH were added; formation of intense yellow colour is observed. To this, few drops 70% dilute HCl were added and yellow colour was disappeared. Formation and disappearance of yellow colour indicates the presence of flavonoids in the extract.

3.Determination of Saponins

To 2 ml of each extract, 6 ml of distilled water were added and shaken vigorously. Formation of bubbles or persistent foam indicates the presence of saponins

4.Determination of tannins

To 1 ml of each extract, 10% of alcoholic ferric chloride was added. Formation of brownish blue or black colour indicates the presence of tannins.

5.Determination of phenols

To 1 ml of each extract, 1ml of 5% aqueous ferric chloride was added. Formation of blue colour indicates the presence of Phenols in the extract.

6.Determination of proteins

To 2 ml of each extract, 1ml of 40% NaOH and few drops of 1% copper sulphate were added. Formation of violet colour indicates the presence of peptide linkage molecule in the extracts.

7.Determination of cardiac glycosides

To 1 ml of each extract, 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added. Formation of brown ring at the interface indicates the presence of cardiac glycosides in sample.

8.Determination of Terpenoids

To 1 ml of each extract, 0.5 ml of chloroform was added followed by a few drops of concentrated sulphuric acid. Formation of reddish-brown precipitate indicates the presence of terpenoids.

9.Determination of carbohydrates

To 1 ml of each extract, few drops of Molisch's reagent were added and 1ml of concentrated sulphuric acid was added by the side of the tubes. The mixture was then allowed it for 2 to 3 minutes. Formation of red or dull violet colour indicates the presence of carbohydrates in the extract.

10.Determination of Quinones

To 1 ml of each extract, few drops of concentrated hydrochloric acid were added. Formation of yellow precipitate or coloration indicates the presence of Quinones.

4.2.12 ANTIMICROBIAL ACTIVITY

Methodology: (Nair. *et al.*, 2005.)

Antibacterial activity of the 2 Tea samples were determined by Agar well diffusion method on Muller Hinton Agar (MHA) (Hi media) plates against pathogens like, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella*. 18 to 24h old culture of the test organism was swabbed onto sterile MHA plates and wells were punched using a 6mm sterile cork borer. 80µl of sample was added in each well along 10mg/ml Gentamycin as +ve control. The sample numbers labeled on the plates are mentioned below. The plates were incubated at 37°C for 24 hours for Bacteria in an incubator. The inhibition was determined by measuring the zones in mm.

4.2.13 ANTIOXIDANT TEST

Antioxidant substances can slow or prevent damage to cells caused by free radicals present in our body. Probiotic can produce more metabolic compounds with antioxidant activity, such as glutathione, butyrate and folate

4.2.14 DPPH Assay (1,1-Diphenyl- 2- picryl hydrazyl)

The solvent extracts of the sample were taken in triplicates in the following concentration range (100, 200, 400, 600, 800 and 1000 μ l) in each test tubes and make up the volume up to 1ml. 3ml of 0.1mM DPPH is added to each test tube. The mixture was shaken and incubated for 30mins in dark and measured at absorbance 517nm in Spectrophotometer. The free radical scavenging activity will be calculated by the formula

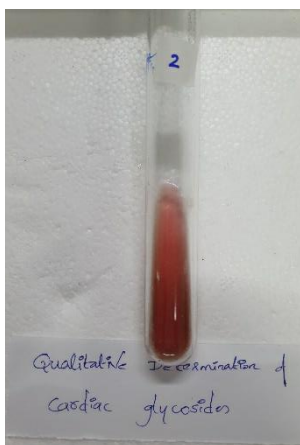
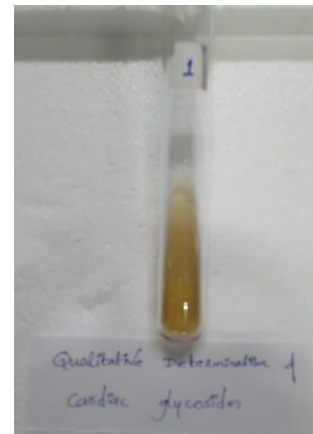
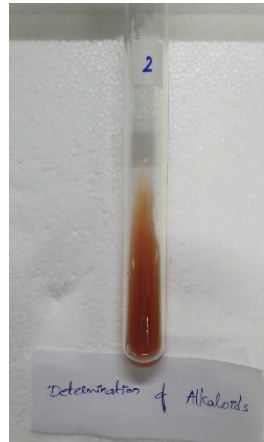
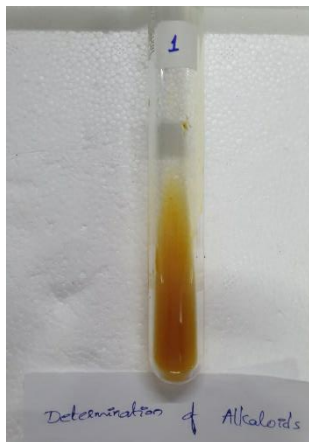
$$\text{Scavenging activity (\%)} = \{(Ad - As)/Ad\} \times 100$$

Where Ad is the absorbance of the alcoholic solution and As is the absorbance of the sample

CHAPTER-5

RESULT AND DISCUSSION

5.1 phytochemical analysis :

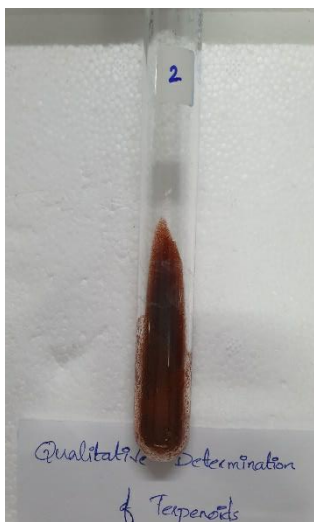
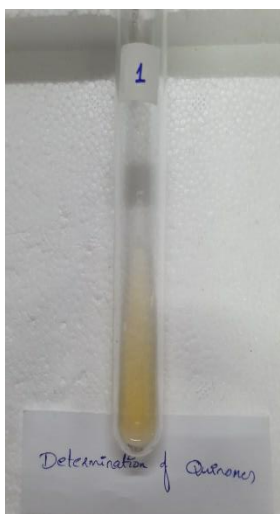


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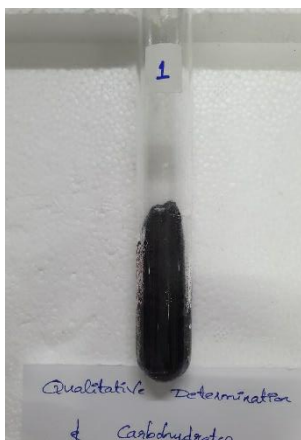


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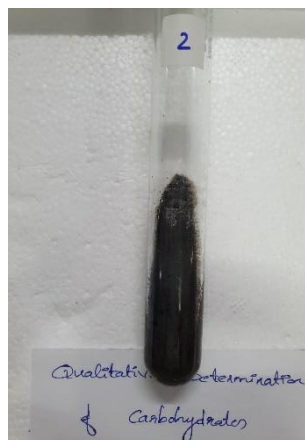
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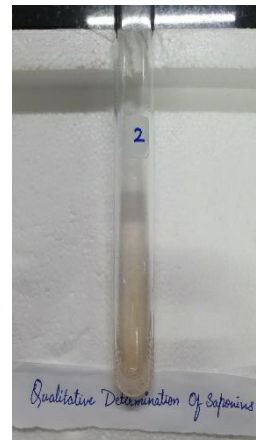
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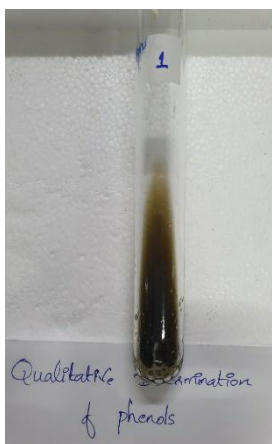
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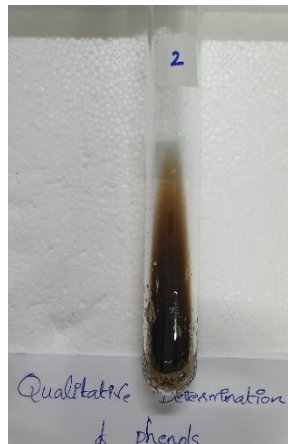
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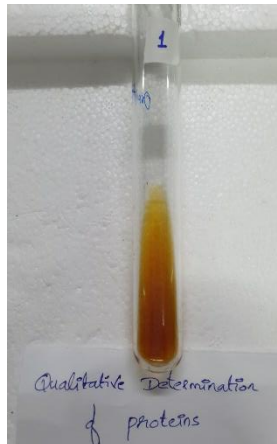
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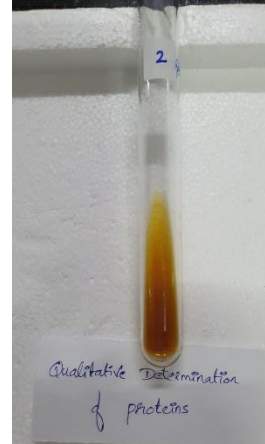
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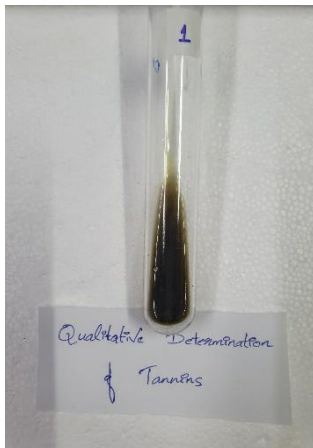
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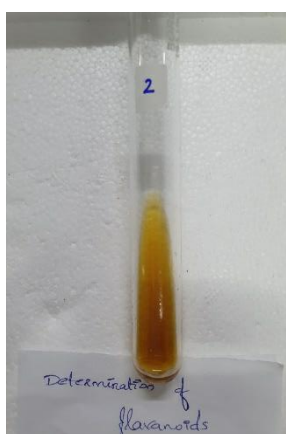
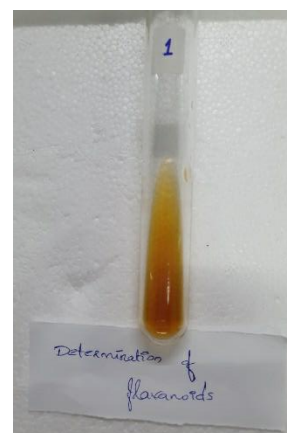
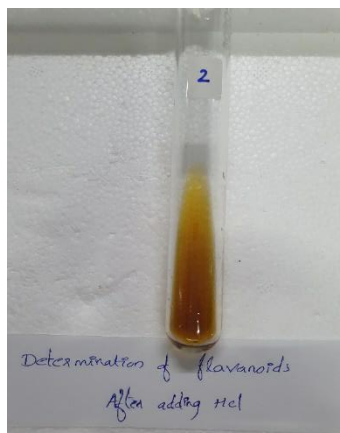
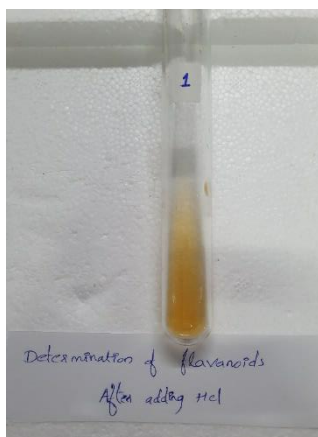


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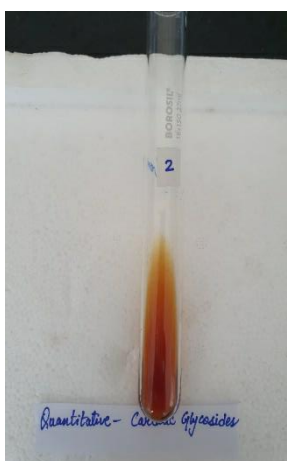
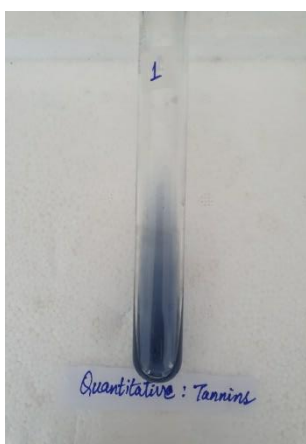
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B(23)

Fig 5.1 results of phytochemical analysis (qualitative) of green tea(Alkaloids ,glycosides, quinones, terpenoids, carbohydrates, saponins, phenols, proteins, tannins, cardiac glycosides, flavonoids after adding HCL, flavonoids)

5.2 QUANATITATIVE PHYTOCHEMICAL ANALYSIS:



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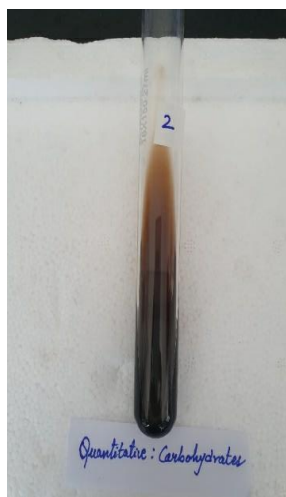
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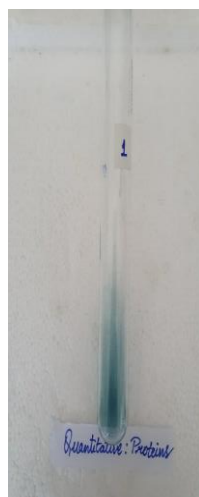
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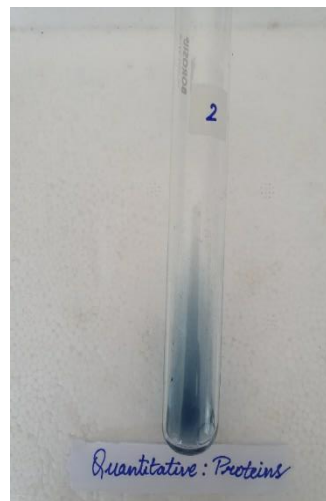
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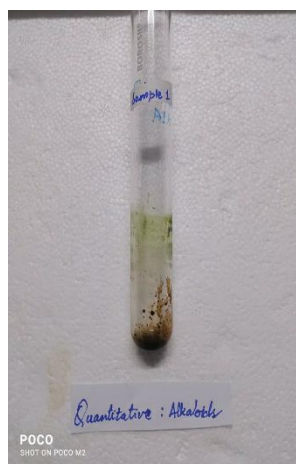
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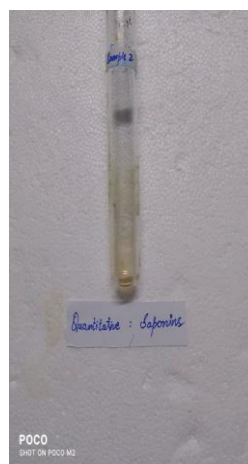
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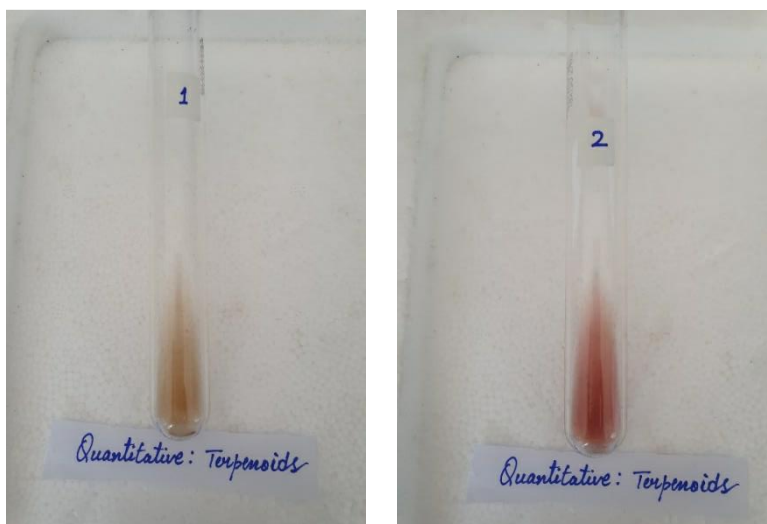
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B(6)



A(6)



A(7)

B(7)

Fig 5.2 results of Phytochemical analysis(Quantitative) of green tea

SAMPLE 1

TABLE 1: QUANTITATIVE ANALYSIS FOR SAMPLE 1

GG	
Test	Concentration (mg/mL)
Tannins	1.14
Proteins	0.10
Carbohydrates	1.32
Cardiac glycosides	0.82
Terpenoids	0.51
Alkaloids	0.11
Saponins	0.14

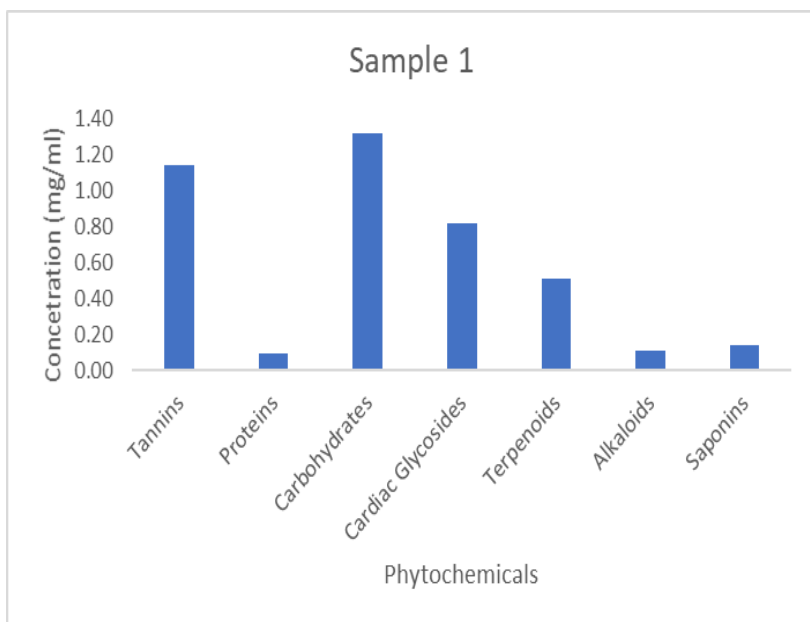


Fig 5.3: QUANTITATIVE ANALYSIS OF SAMPLE 1(mg/ml)

Sample 2

TABLE 2: QUANTITATIVE ANALYSIS FOR SAMPLE 2

Sample 2	
Test	Concentration (mg/mL)
Tannins	0.9
Proteins	0.1
Carbohydrates	1.5
Cardiac glycosides	0.98
Terpenoids	1.07
Alkaloids	0.12
Saponins	0.1

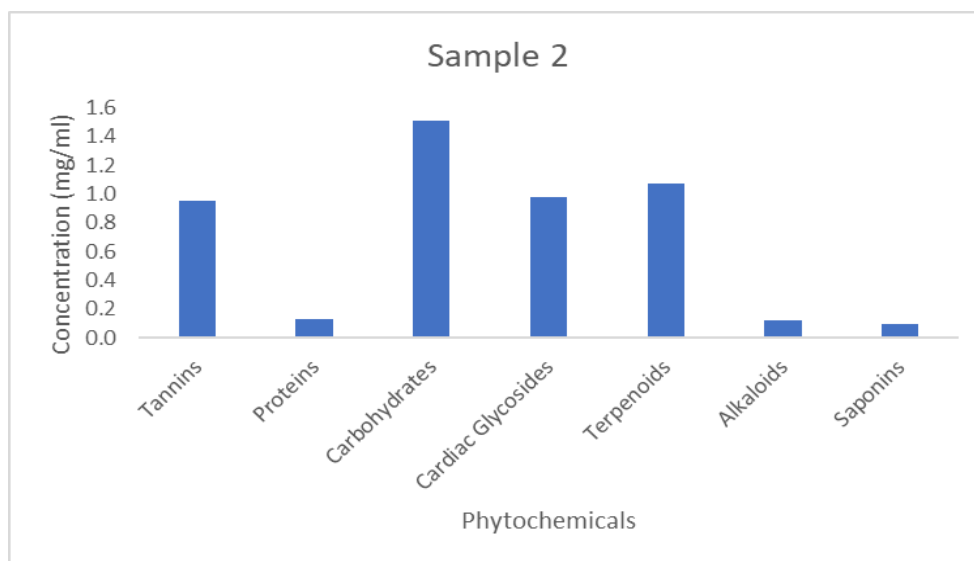


Fig 5.4: QUANTITATIVE ANALYSIS OF SAMPLE 2(mg/ml)

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5.3 PROTOCOLS AND STANDARD GRAPH

5.4 TOTAL TANNINUS CONTENT DETERMINATION:

The tannins were determined by little modified Folin and Ciocalteu method. 1 ml of sample extract is added with 7.5 ml of distilled water with add 0.5 ml of Folin Phenol reagent and 1 ml of 35% sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions (0 to 0.5mg/ml) were used as standard solutions. The results of tannins are expressed in terms of tannic acid in mg/ml of extract.

TABLE 3: STANDARD FOR TANNINS

Sample	OD @ 765nm	Tannins (mg/ml)
Standard1	0	0
Standard2	0.2	0.1
Standard3	0.4	0.23
Standard4	0.6	0.35
Standard5	0.8	0.49
Standard6	1	0.6

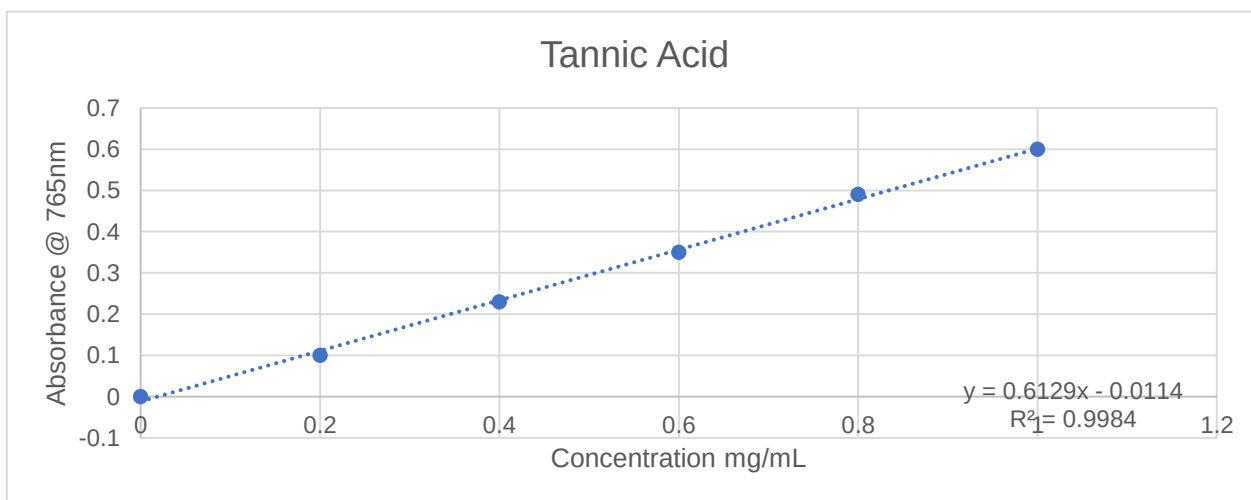


Fig.5.5 Standard curve of tannic acid (mg/ml)

5.5 Total Protein content determination:

The entire proteins content of the sample was determined by using Bradford's method. To 100 μ l of the sample extract add 3ml of Bradford's reagent and incubate in dark for 5mints. The absorbance was measured at 595nm.

TABLE 4: Standard for Proteins

Sample	OD @ 765nm	Proteins (mg/ml)
Standard1	0	0
Standard2	0.2	0.695
Standard3	0.4	1.187
Standard4	0.6	1.659
Standard5	0.8	2.33
Standard6	1	2.973

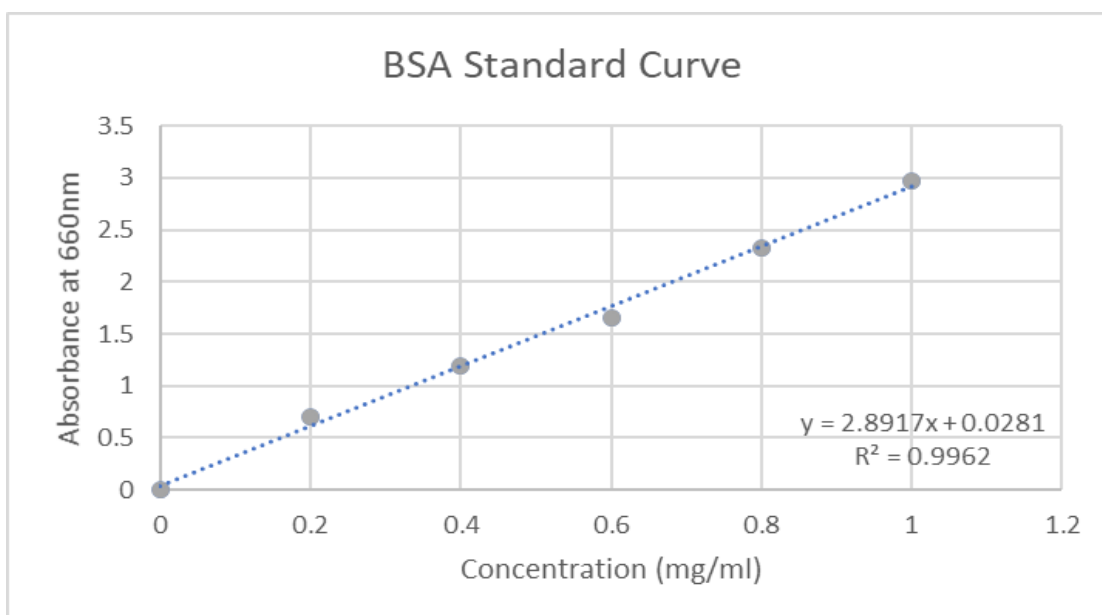


Fig:5.6 STANDARD CURVE OF BOVINE SERUM ALBUMIN (mg/ml)

5.6Total Flavonoids Content Estimation: (P. K. Ramamoorthy and A. Bono, 2007)

5ml of 2% $AlCl_3$ prepared in methanol was mixed with same volume and mixed with same volume of extract solution. After 10 min, absorbance was taken at 415nm against blank. Blank was prepared as 5 ml of extract mixed with 5 ml of methanol without $AlCl_3$. Catechin was used to prepare standard graph.

Table 5: Standard for Proteins:

Sample	OD @ 765nm	flavanoid(mg/ml)
Standrad1	0	0
Standrad2	0.02	0.018
Standrad3	0.04	0.035
Standrad4	0.06	0.059

Standrad5	0.08	0.075
Standrad6	0.1	0.097

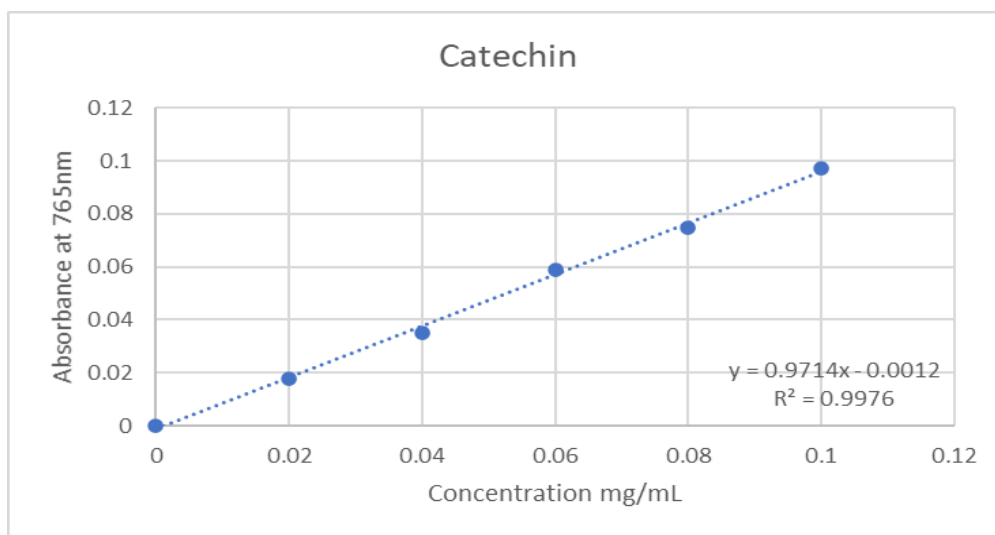


Fig.5.7 STANDARD CURVE OF CATECHIN (mg/ml)

5.7 Total Alkaloid content determination: (Gracellin et al., 2013)

40 ml of 10% acetic acid in ethanol was added to 1g of powdered sample, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle; collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

5.8 Total Carbohydrate determination:

For estimating the polysaccharide content, add 1ml of 5% phenol to the 1ml of sample solution, and then add 5ml of concentrated H₂SO₄ and measure the absorbance after 10 minutes at 488nm against blank. To prepare Blank, 1ml of distilled water added to 1ml of 5% phenol followed by 5ml of Concentrated H₂SO₄.

Table 6: Standard for Carbohydrates:

Sample	OD at 488nm	Carbohydrates mg/mL
Standrad1	0	0
Standrad2	0.02	0.312
Standrad3	0.04	0.462
Standrad4	0.06	0.727
Standrad5	0.08	1.015
Standrad6	0.1	1.313

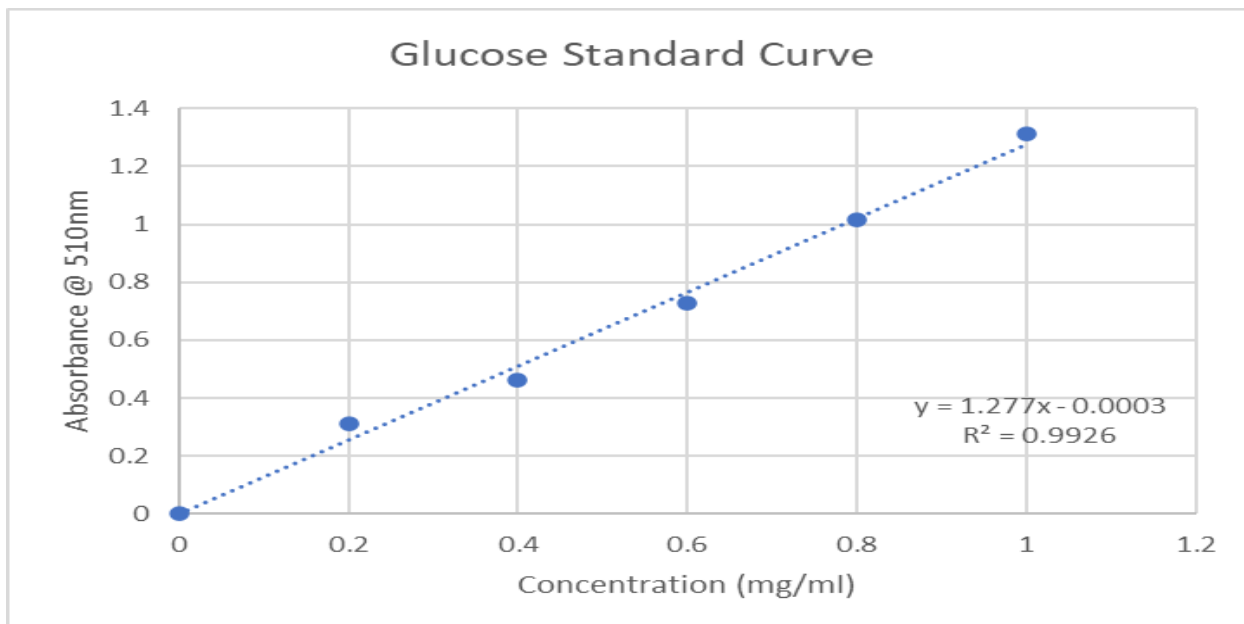


Fig 5.8 STANDARD CURVE OF GLUCOSE (mg/ml)

5.9 Determination of saponins: (Obadoni and Ochuko, 2001)

2 g of each sample was dispersed in 20ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 20ml of 20 % ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. The concentrate was transferred into a 250ml separatory funnel and 2ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the purification process was repeated. 6ml of n-butanol was added. The combined n-butanol extracts were washed twice with 1ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act

primarily against. As an example, antibiotics are mostly used against bacteria and antifungals are used against fungi. They can also be classified according to their function.

5.10 ANTIMICROBIAL ACTIVITY

Methodology: (Nair. *et al.*, 2005.)

Antibacterial activity of the 2 Tea samples were determined by Agar well diffusion method on Muller Hinton Agar (MHA) (Hi media) plates against pathogens like, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella*. 18 to 24h old culture of the test organism was swabbed onto sterile MHA plates and wells were punched using a 6mm sterile cork borer. 80µl of sample was added in each well along 10mg/ml Gentamycin as +ve control. The sample numbers labeled on the plates are mentioned below. The plates were incubated at 37°C for 24 hours for Bacteria in an incubator. The inhibition was determined by measuring the zones in mm.



Fig 5.9 antimicrobial analysis of sample 1 and 2

TABLE 7:Zone of inhibition for bacteria

	Staphylococcus aureus	Escherichia coli	Klebsiella pneumoniae	Bacillus Subtilis	Salmonella
Sample names	Zone of Inhibition (mm)				
1	12	00	13	10	00
2	11	00	10	09	00
+ve Control	27	24	26	31	11

5.11 ANTIOXIDANT TEST

Antioxidant substances can slow or prevent damage to cells caused by free radicals present in our body. Probiotic can produce more metabolic compounds with antioxidant activity, such as glutathione, butyrate and folate

5.12 DPPH Assay (1,1-Diphenyl- 2- picryl hydrazyl) (Hemalatha *et al.*, 2015)

The solvent extracts of the sample were taken in triplicates in the following concentration range (100, 200, 400, 600, 800 and 1000µl) in each test tubes and make up the volume up to 1ml. 3ml of 0.1mM DPPH is added to each test tube. The mixture was shaken and incubated for 30mins in dark and measured at absorbance 517nm in Spectrophotometer. The free radical scavenging activity will be calculated by the formula

$$\text{Scavenging activity (\%)} = \{(Ad - As)/Ad\} \times 100$$

Where Ad is the absorbance of the alcoholic solution and As is the absorbance of the sample

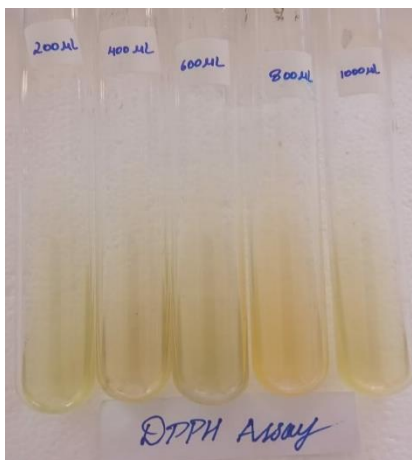


Fig 5.10 DPPH assay of sample 1 (at different concentrations (200 µl, 400 µl, 600 µl, 800 µl, 1000 µl)).

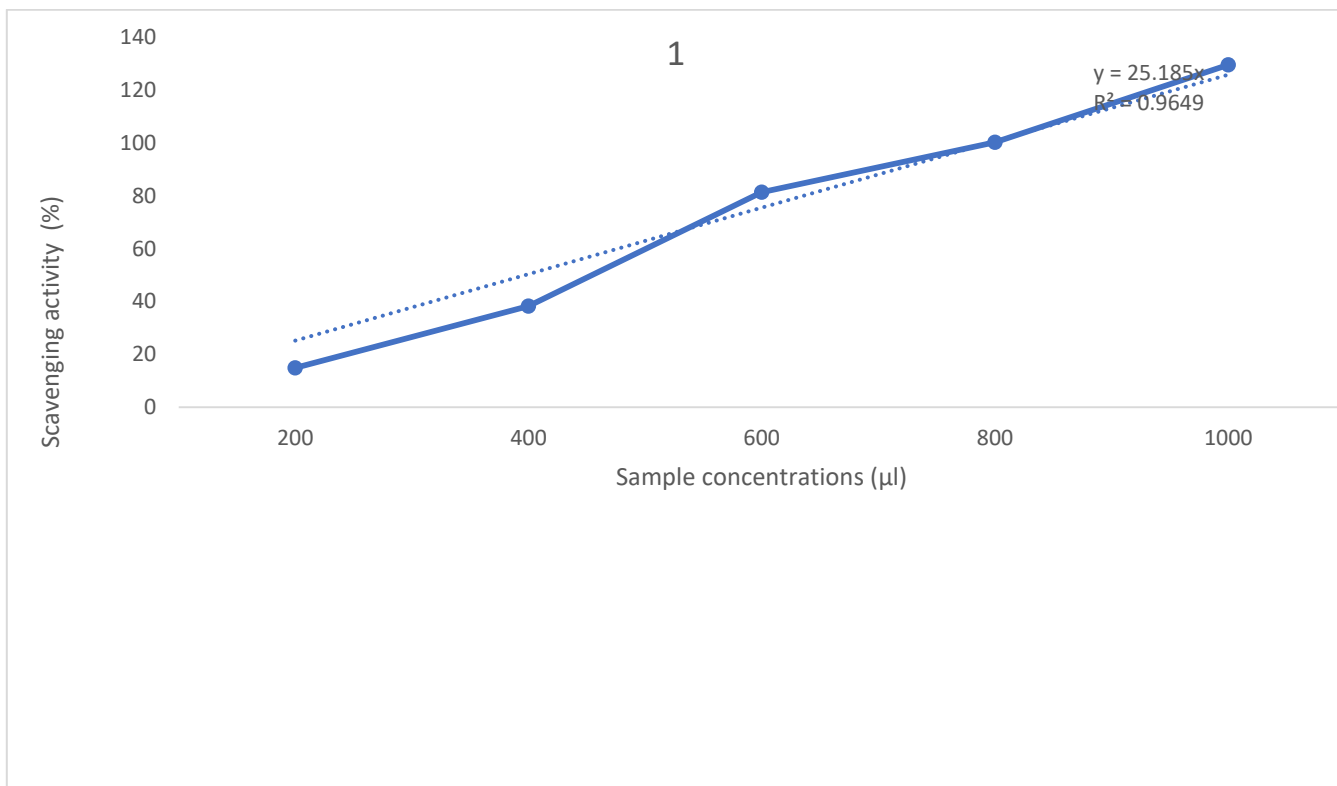


Fig.5.11 Scavenging activity of sample 1

Table:8 Scavenging activity (%) of the sample - 1

Concentrations (μl)	Absorbance at 517 nm	Scavenging Activity (%)	IC50
	1GM		
200	0.149	14.9	1.9
400	0.382	38.2	
600	0.814	81.4	
800	1.003	100.3	
1000	1.297	129.7	

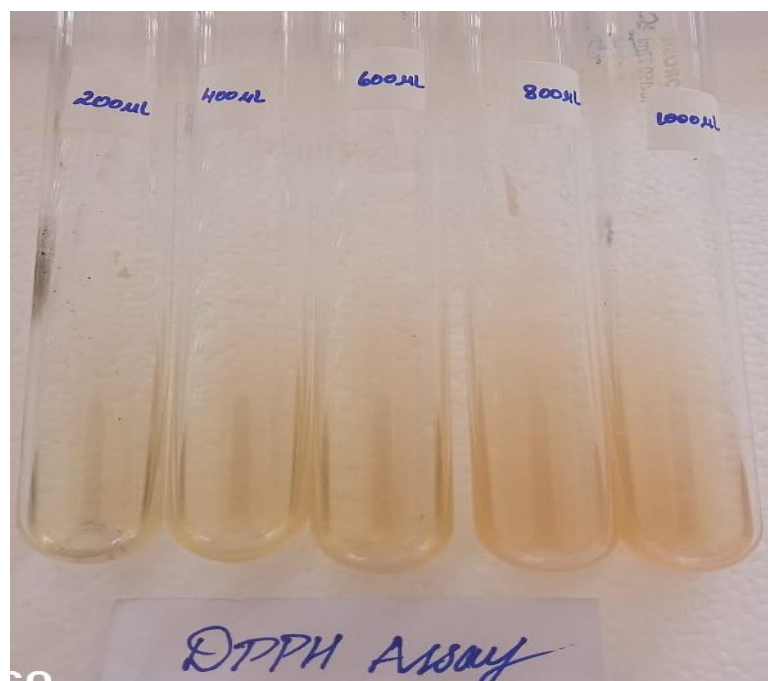


Fig.5.12 DPPH assay of sample 2 (at different concentrations (200 μ l, 400 μ l, 600 μ l, 800 μ l, 10

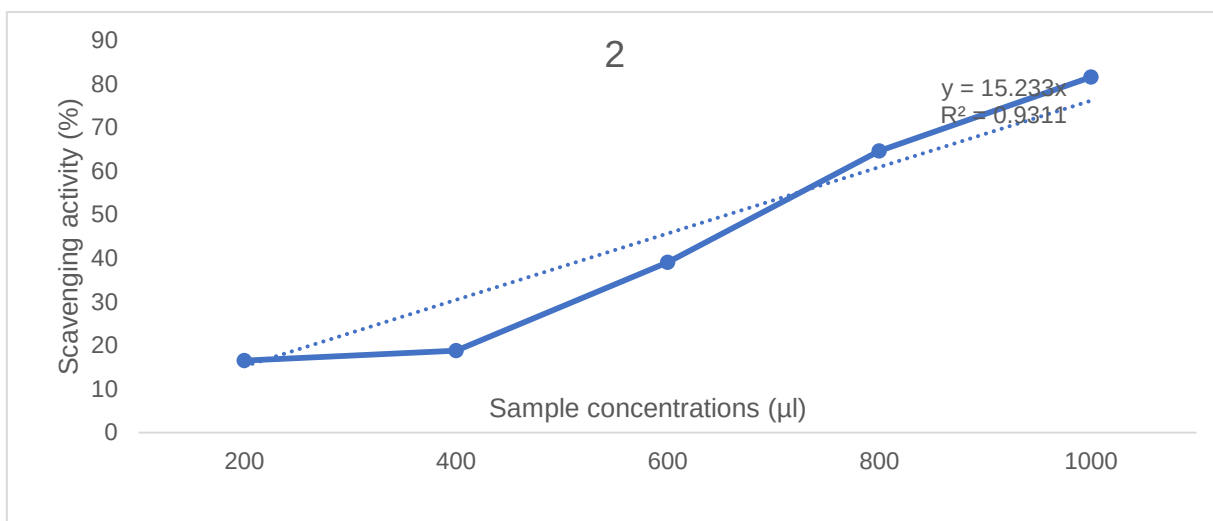


Fig.5.13 Scavenging activity of sample 2

Table 9. Scavenging activity (%) of the sample - 1GF

Concentrations (µl)	Absorbance at 517 nm	Scavenging Activity (%)	IC50
	1GF		
200	0.165	14.9	3.3
400	0.188	38.2	
600	0.391	81.4	
800	0.646	100.3	
1000	0.816	129.7	

CHAPTER -6

SUMMARY AND CONCLUSION:

Green tea is considered as one of the healthiest options. It was a part of Asian culture to consume tea on a regular basis, which can give a great kick start to a wonderful morning. Consuming it regularly can improve overall metabolism and well-being of humans. Tea is one of the versatile options available in the market right now, and it can be very tasty and healthy. To have a warm cup of tea anytime of the day, green tea has many more health benefits. A cup of tea has very few calories and is packed with tons of nutrients and healthy components, which makes it ideal for health-conscious people. As well, drinking a plain and bland tea cannot be much appetizing sometimes, so we have an alternative for that by adding natural flavors to the tea. Not just enhancing its taste but also enhancing its nutrient values, it can be a great alternative if not the best alternative for other soft drinks available in the market.

CHAPTER 6

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