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Optimization of the Extraction of Polyphenols from Green Tea (*Camellia sinensis)*

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Abstract: **Tea, the most popular beverage consumed is widely cultivated in India and China. Of the different kinds of Tea about 20% of Green Tea is being harvested annually. Green tea has gained popularity due to its remarkable health benefits. People who have consumed Green Tea were less likely to develop type-II diabetes. About 200 bioactive compounds are present in Green Tea of which,the most active are the polyphenols. Green Tea has 8000 polyphenolic compounds of which EGCG(Epigallocatechingallate) is more important (8-12% of total polyphenols). The present study focus to extract maximum amount of polyphenol by Folins Ciocalteau method for optimizing the extraction time and temperature and further utilize it to prevent diabetes. Aqueous extraction of Green Tea polyphenols was performed at three different temperatures 40°C, 50°C and 60°C and at three corresponding time 15 minutes, 30 minutes and 60 minutes. From the present study, maximum polyphenols content was obtained at 40°C for 30 minutes.**

Keywords: **Polyphenol, EGCG, Extraction, Bioparameters, Green Tea.**

1. INTRODUCTION

Polyphenols, the secondary metabolite are naturally occurring bioactive compounds largely found in fruits, vegetables, cereals and beverages and are involved in plant's defence mechanism. In food polyphenols contribute to bitterness, astrigency, colour, flavour, odour and oxidative stability. Several different types of polyphenolic compounds with antioxidant properties in Green Tea have been identified, with the most predominantly active component - flavanol known as catechins[1][2][3]. Of the five various Catechins , Epigallocatechin-3-gallate(EGCG) is the most effective compound. This is known to interfere with glucose homeostasis, carbohydrate metabolism, LDL, cholesterol, indirectly involved in lipogenesis and hence the risk of obesity decreases[4] . It has been demonstrated that body weight of rats and their plasma triglyceride, cholesterol, LDL levels have been reduced by feeding Green Tea[5]. For this reason Green Tea is preferred by people worldwide to manage weight loss. Also EGCG in Green Tea helps to sensitize cells which metabolize sugar thus regulating blood glucose level. Green Tea does not completely inhibit the glucose absorption from small intestine instead it slow downs its absorption rate. This is an indicative measure of using polyphenol in prevention of type II diabetes.

Medicinal plants like Milk thistle, Guggul, Banaba leaves, Cinnamon, Garlic, licorice, Green Tea act directly on pancreas and stimulate insulin level in blood. Green Tea polyphenols are also responsible for distinctive aroma, color and taste. Green Tea extract can also be used in lipid-bearing foods to delay lipid oxidation and to enhance the shelf-life of various food products. It also helps in preventing several autoimmune diseases such as rheumatoid arthritis. The extraction and purification process of polyphenols is simple because it is highly polar in nature, thus water can be used as a solvent. The product obtained is less toxic with no side effects, hence Green Tea can be supplemented as health promoter. Our aim is to find out the maximum yield of polyphenols by modifying Green Tea formulations.

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2. MATERIALS USED

Glass Wares**,** 1% Ferric Chloride**,** Folin's Ciocalteau reagent**,** 7.5% Sodium bicarbonate, Magnetic stirrer, Micropipette, Spectrophotometer.

3. METHODS

3.1. Sample preparation:

Dried Green Tea leaves were procured from Biolim centre for Science & technology (BCST) organization. These leaves were then grounded to powder using Mixer.

3.2. Choice of solvent:

Water is found to be an excellent solvent for extracting polyphenol as water is cost effective, non toxic and gives maximum yield.

3.3. Aqueous extraction:

2g of Green Tea powder was weighed and dissolved in 100ml of distilled water in a beaker. This concept is then subjected to Magnetic stirrer for various temperature and time period[6]. It is then filtered using Whatmann filter paper no.1 and the sample extracted is used for further analysis (fig 1).

3.4. Qualitative test for phenols:

Ferric chloride test for phenols is the method employed to estimate phenol qualitatively. To 1ml of the plant extract add 20µl of 1% ferric chloride solution. The appearance of bluish black colour precipitate (fig 2) indicates the presence of phenols[7].

3.5. Folin's Ciocalteu assay for phenol estimation:

The principle used in this method is the oxidizing property of the reagent which is a mixture of phosphotungstic and phosphomolybdic acid that are reduced by the oxidation of phenolic compounds. 0.2ml of the test sample is mixed with 1ml of Folin's Ciocalteu stock and left for 10 minutes. Add 0.8ml of sodium bicarbonate solution, and place it in dark for 30 minutes[8]. The reaction turns the sample blue in colour depending upon the phenolic content in them (fig 3). This test is performed in triplicates. Gallic acid is used as a reference standard and optical density is read against blank. The amount of polyphenols obtained is calculated using the formula:

Concentration (µg) = Unknown absorbance / Standard absorbance

4. RESULTS AND DISCUSSION

Fig 1: Different extraction is performed at variable time and temperatures

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Fig 2: Qualitative test for phenol

When 1% Ferric chloride was used the colour changes to bluish black which confirms the presence of phenol.

Fig 3: Quantitative estimation of total phenolic content using Folin's Ciocalteau method

By using Folin's Ciocalteau reagent, samples turn blue in colour depending on the amount of phenolic content present.

2% of the Green Tea extract at various concentrations and temperature for 15 minutes:

Table 1: Extraction at various temperature for 15 minutes.

S.No	Volume (µl)	Average OD at 700 nm			Time (minutes)	Concentration (µg/ml)		
		40˚C	50˚C	60˚C		40˚C	50˚C	60˚C
1	20	0.135	0.54	0.21		10.38	41.53	16.15
2	40	0.18	0.66	0.37	15	13.84	50.76	28.46
3	60	0.27	0.83	0.57		20.76	63.84	43.84
4	80	0.29	0.88	0.77		22.30	67.69	59.23
5	100	0.39	1.11	0.92		30.00	**85.38**	70.76

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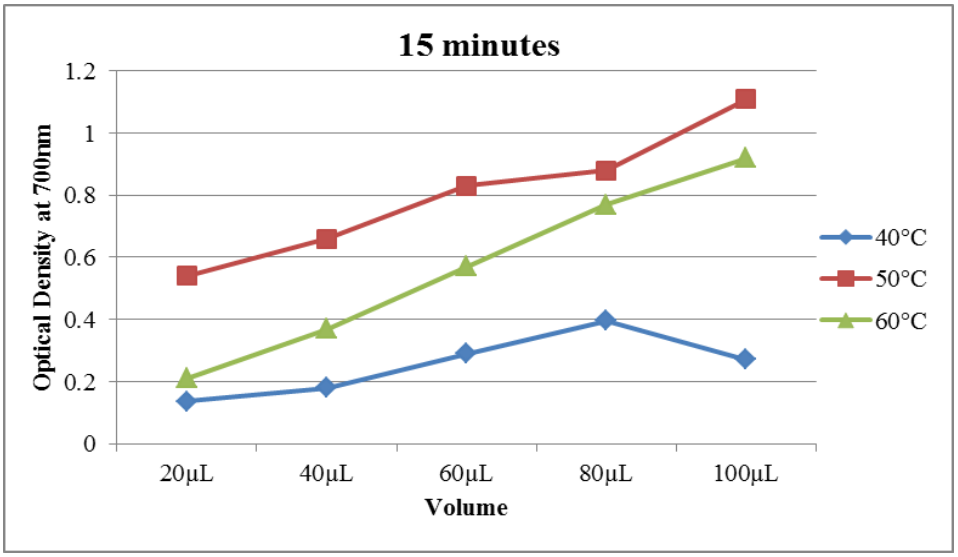

Graph 1: Different absorbance values for 15 minutes extraction at 40°C, 50°C and 60°C.

The amount of polyphenols in 2% of the Green Tea extract was found to be maximum at 50°C for 15 minutes. The polyphenols varied using different temperature at constant time. The maximum amount of polyphenols obtained was 85.38µg in 100µl of the sample. The total amount of polyphenols present in 100ml of the extract (50°C, 15 minutes) was 0.08538 mg. The total yield obtained is **8.5%.**

2% of Green Tea extract at various concentrations and temperatures for 30 minutes:

Table 2: Extraction at various temperature for 30 minutes

S.No	Volume (µl)	Average OD at 700 nm			Time (minutes)	Concentration (µg)		
		40°C	50°C	60°C		40°C	50°C	60°C
1	20	0.65	0.49	0.42		50.00	37.69	32.30
2	40	0.93	0.695	0.55		71.53	53.46	42.30
3	60	1.11	0.82	0.68	30	85.38	63.07	52.30
4	80	1.45	1.16	0.83		111.53	89.23	63.84
5	100	1.62	1.29	0.98		**124.6**	99.23	75.38

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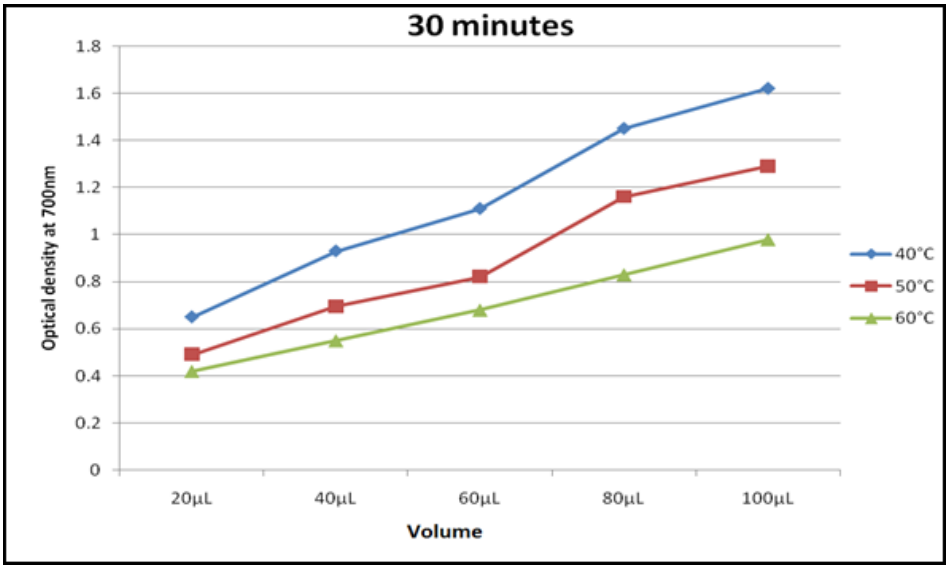

Graph 2: Different absorbance values for 30 minutes extraction at 40°C,50°C,60°C

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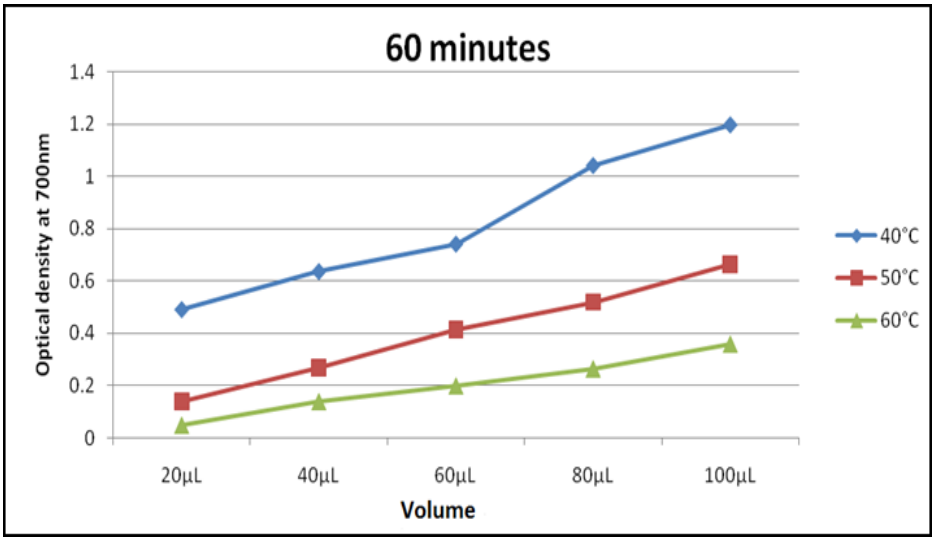
The amount of polyphenols in 2% of Green Tea extract was found to be maximum at 40°C for 30 minutes. 124.6 µg was obtained in 100µl and therefore the total polyphenols present in 100ml of the extract (40°C, 30 minutes) was 0.1246 mg. The total yield obtained is **12.4%.**

2% of the Green Tea extract at various concentrations and temperatures for 60 minutes:

Table 3: Extraction at various temperature for 60 minutes

S.No	Volume (µl)	Average OD at 700 nm			Time (minutes)	Concentration (µg)		
		40°C	50°C	60°C		40°C	50°C	60°C
1	20	0.49	0.14	0.05		37.69	10.76	3.84
2	40	0.63	0.27	0.14		48.46	20.76	10.76
3	60	0.74	0.41	0.20	60	56.92	31.53	15.38
4	80	1.04	0.52	0.26		80.00	40.00	20.00
5	100	1.19	0.66	0.36		**91.53**	50.76	27.69



Graph 3: Different absorbance values for 60 minutes extraction at 40°C,50°C,60°C

The amount of polyphenols in 2% of Green Tea extract was found to be maximum at 40°C for 60 minutes . 91.53µg is the maximum amount obtained in 100µl of the sample and therefore total polyphenols in 100mL of the extract (40°C, 60 minutes) was 0.09153 mg. The total yield obtained is **9.1%.**

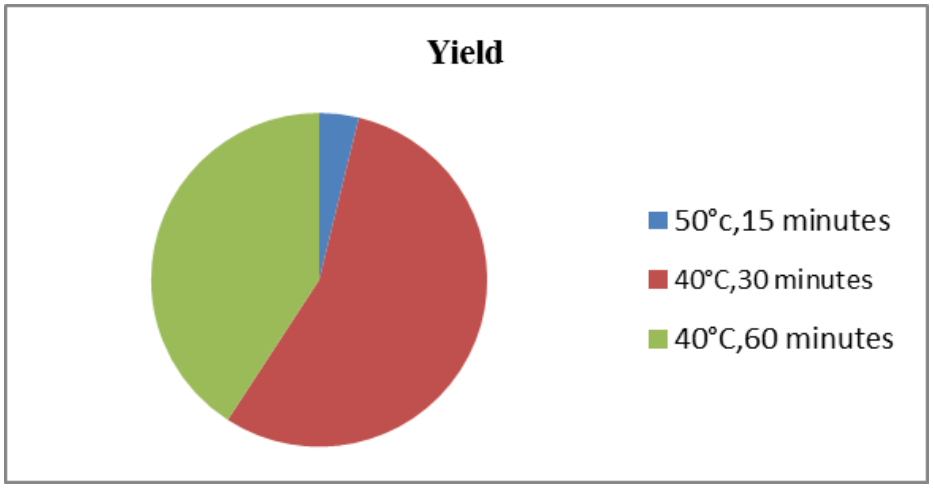


Fig 4: Comparative analysis of polyphenols at varying Bio parameters

From the above figure, it can be inferred that the activity of polyphenol was maximum at 40°C for 30 minutes.

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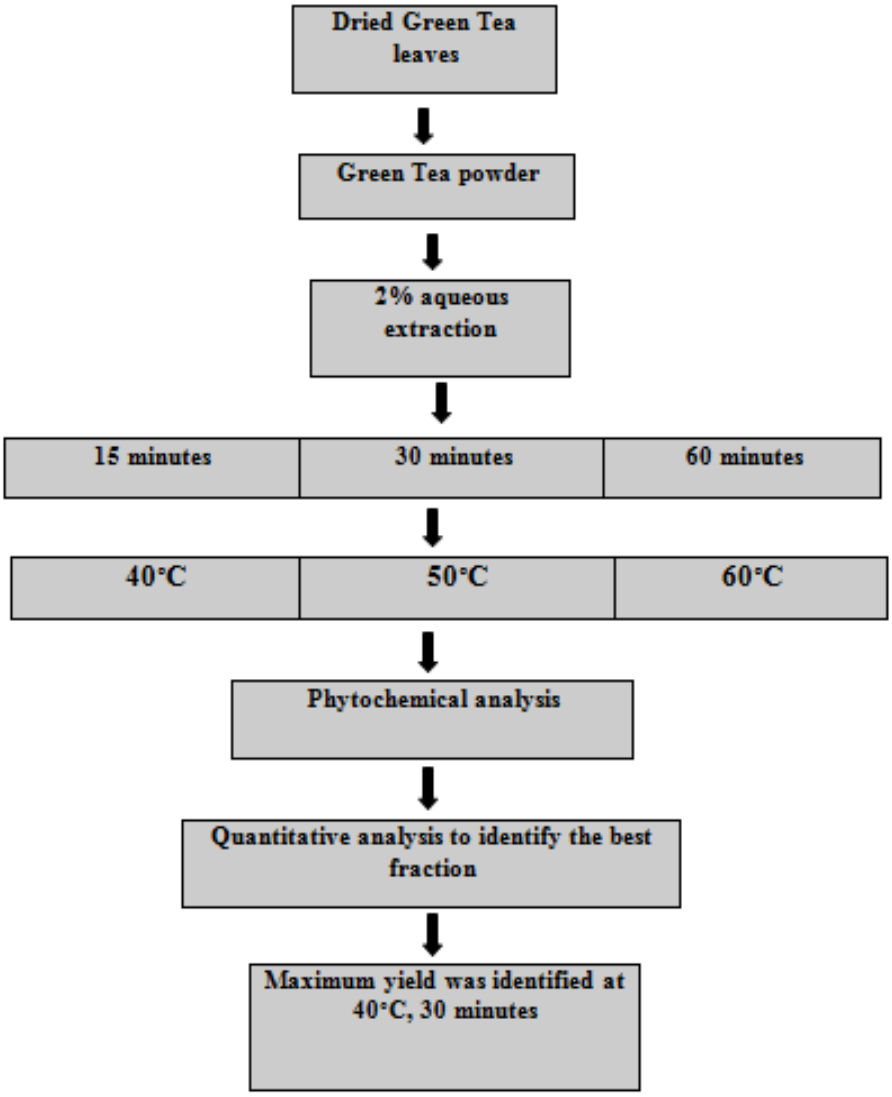

Fig 5: Schematic representation of extraction of polyphenols from Green Tea

5. CONCLUSION

Aqueous extraction of Green Tea polyphenols was performed at three different temperatures 40°C, 50°C and 60°C and at three corresponding time 15 minutes, 30 minutes and 60 minutes. Maximum polyphenols content was obtained at 40°C for 30 minutes. Based on these studies it is concluded that as the concentration of sample is increased, the activity of polyphenol is also increased and as the temperature increases activity decreases. Therefore the concentration of the extract is inversely proportional to temperature. Time and temperature is found to play a significant role on the activity of polyphenol.

6. FUTURE PROSPECTS

- Study the mechanism of anti-diabetic activity of Green Tea polyphenols
- Drug formulation for oral administration

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REFERENCES

[1] J.Perez J,S Arranz,M Tabernero et al,methodology to determine antioxidant capacity in plant food,oils,food res Int,vol-41,2008:274-285

[2] G.Miliavskas,PR Venskutonins,TA Van beer,screening of radical scavenging activity of some medicinal and aromatic plant extracts,foodchem,vol 85,2004,pp 231-237

[3] MH Abdille,RP Singh,GK Jayakrishna, BS Jena,antioxidant activity of extracts from Dilenia indica fruits, food chem, vol 90,issue 4,2005,pp-891-896

[4] Megan.W,green tea;health benefits,fats and research,2016,released in medicalnewstoday.

[5] Ronald H,EGCG;potent extract of green tea,realeased on October 4 2013 in the newsletter intelligent medicine.

[6] [6]. Muhamahad HA,Taha r,Khalil e,optimisation extraction conditions for phenolic compounds,journal of food biochemistry,dec 2015,pp121-128

[7] Kumar S B, Kumari S N, Vadisha S B, Sharmila K P & Prasad M B. Preliminary phytochemical screening of various extracts of Punica granatun peel, whole fruit and seeds. Journal of Health Science.2012.2 (4):34-38.

[8] Cavalcanti de Amorim E L, Nobre de Almeride de Castro V T, Aguilar-Sanchez R, Garciat A, Davis-Jimenez, Elizalde-Gonnzalez M M & Guevare-Villa M R. Chromatographic and electrochemical determination of quercetin and kaempferol in phytopharmaceuticals. Journal of Pharmaceuticals and Biomedical analysis.2005.38 (2):239-249.