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Extraction, Evaluation and Comparison of Antioxidant Properties of Darjeeling Tea (Tata Tea) and Filter Coffee (Malgudi Coffee)

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ABSTRACT

The present study into evaluate the antioxidant property of Darjeeling Tea and Filter Coffee extract using decoction method. The project involves determining the percentage yield of the extract, phytochemical screening and accessing the total phenolic content and antioxidant assay of the extract. This result indicated that coffee has higher anti oxidant activity than tea. In conclusion the evaluation of antioxidant property of Darjeeling Tea and Filter Coffee reveals both extract contains a variety of phytochemical with antioxidant potential. The evidence are total phenolic content, Hydrogen Peroxide scavenging activity and phosphomolybdate reagent assay.

KEYWORD – Darjeeling Tea, Filter Coffee, Hydrogen peroxide, phosphomolybdate, total phenolic content.

Introduction

Antioxidants are the compounds that inhibit oxidation. Antioxident are substances that reduce damage due to oxygen such as free radicals. Well known antioxidents are Vitamin C, Vitamin E and Bita Carotene, which can counter act the damaging effect of oxidation. Anti oxidant also slows down the process of age related macular degeneration. This anti oxidant have remarkable property to combact free radical thus delaying cellur damage, hence are known as radical scavenger. The importance of anti oxidant is best known in Bio Chemistry of living organism and for identification as reducing agent. In nature, there is a wide Varity of naturally occurring anti oxidant which are different in composition, physical and chemical properties, mechanism and site of action(1

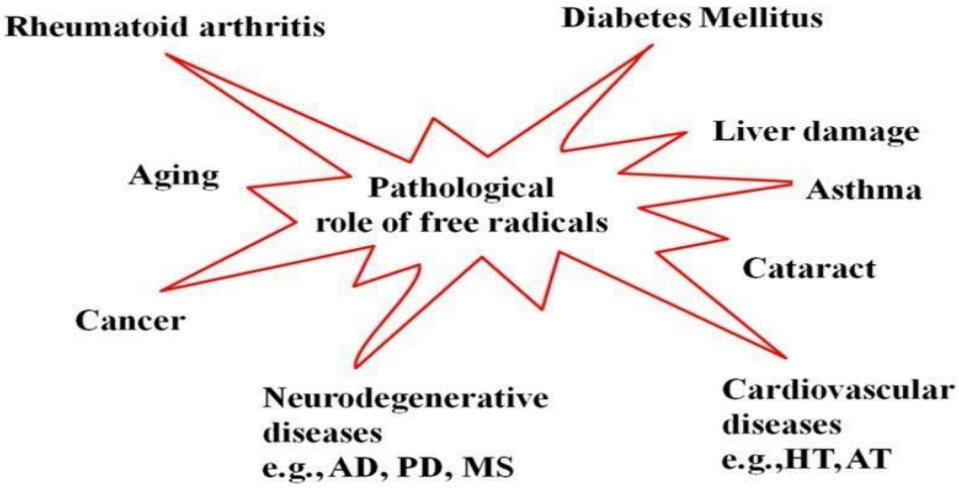

Fig 5:-Pathological role of free radicals

ANTIOXIDANTS- antioxidants are a diverse group of compounds that play a crucial role in maintaining the balance between oxidative stress and body defence mechanisms. They are essential for neutralizing harmful free radicals and prevent oxidative damage to cells and tissues. Antioxidants are naturally present in various food , plants, and even produced within the human body. numerous studies have suggested that diet high in antioxidant rich foods such as fruits, vegetables, whole grains, nuts, may have protective effects against conditions like cardiovascular disease, cancer, neurodegenerative disorders.

Mechanism of action:

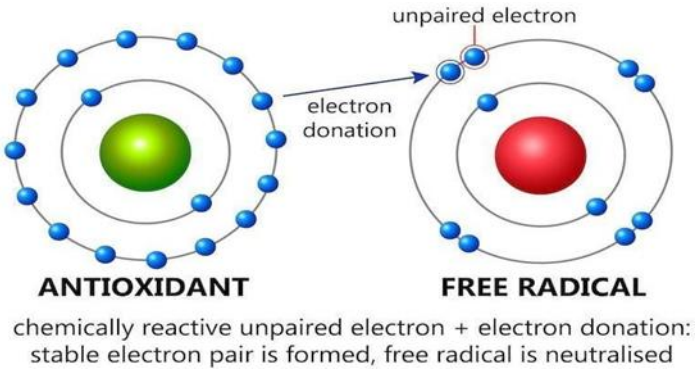

Fig 4:- Mechanism of Antioxidant and free radical

Sources of antioxidants:

. allium sulphur compounds- onion and garlic

. anthocyanins – eggplant, grapes, berries

. betacarotene-pumpkin, mangoes,apricot,carrot, spinach

. catechins- red wine, tea

. copper- seafood,lean meat, milk, nuts.

. flavonoid- tea, citrus food, red wine, apples.

. indoles- broccoli, cabbage.

. lignans- sesame seeds, whole grains, vegetables

. lycopene- tomatoes,watermelon

. selenium- seafood, whole grains.

Types of antioxidants:

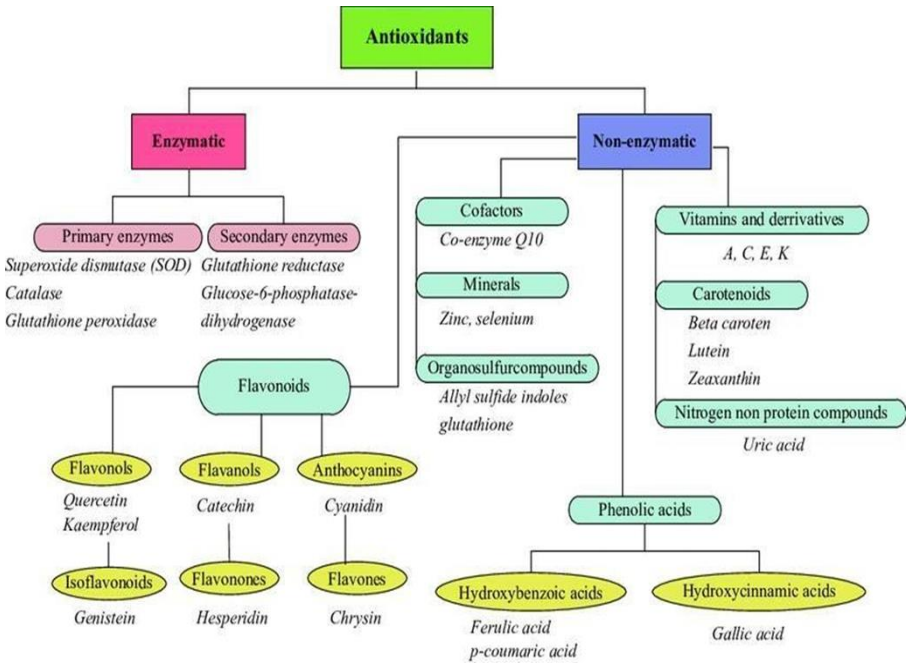

Fig 3:- Types of Antioxidants

METHODOLOGY- there are various methods for extraction of antioxidants:

1) Physical extraction - cold extraction method is one of the common method.it allows extraction of antioxidants rich inner liquid of fruits. It is one of the first step recovery of antioxidants compound from red fruits.

2) Solvent extraction - traditionally two main types-maceration and solvent extraction. Maceration is the extraction of substances from the matrix by the release of them into solvent without heat application for longer period of time. It is used by some researcher to obtain extract rich in antioxidants, whereas soxhlet extraction uses heat application and variety of solvents allows extraction of target components in shorter time.

3) Ultrasound assisted extraction - it is a non thermal technique which uses frequencies equal to 20 khz. The mechanism follows-utrasound induces cavitation which causes cell wall disruption. This allows permeation of intra-cellular compounds and therefore liberate antioxidant.

4) Microwave assisted extraction – it is characterized by generally low or lack of solvent. The intrinsic moisture of fruit is used therefore mass and heat transfer takes in the same direction. Cells are damaged an intra cellular content is released to the medium.

5) Pressure assisted extraction – these are green alternative method that can be classified into pressurized liquid extraction and supercritical CO2 extraction

    a. Pressure liquid extraction: it combines conventional solvent exraction with pressure application. It allows operation with high temperature while solvent remains liquid, enhancing solubility and kinetics.

    b. Super critical CO2 extraction: **:** Supercritical CO2 extraction (SFE-CO2) is a nonconventional extraction technique that operates at very high temperature and pressure (supercritical conditions). This enables high mass transfer rates, difficult to achieve with liquid solvents.

6) Pulsed Electric Fields: Pulsed Electric Fields (PEF), the sample is placed between two electrodes, and an electric field is applied in a pulsed way. Pulse amplitude ranges from 100 V/cm to 80 kV/cm and extraction times of less than a second, in repetitive cycles. This electric field causes damage on plant cell walls, which is known as 'electroporation'. The formed pores allow the release of intracellular compounds into the liquid.

INVITRO STUDIES FOR ANTIOXIDANT PROPERTY:

Introduction:

In vitro studies for evaluating the antioxidant property of substances have become essential in the field of biomedical research. Antioxidants play a crucial role in preventing or reducing oxidative stress, which is associated with various diseases and aging processes. In vitro models provide a controlled environment to investigate the antioxidant capacity of compounds and assess their potential therapeutic applications. This document provides a detailed introduction to in vitro studies for evaluating the antioxidant property, including the importance of antioxidants, commonly used assays, and the significance of in vitro findings.

Importance of Antioxidants: Antioxidants are substances that can neutralize or inhibit the harmful effects of reactive oxygen species (ROS) and free radicals, which are generated during normal cellular metabolism or in response to external factors such as pollution, radiation, or toxins. ROS and free radicals can cause oxidative damage to cells, leading to cellular dysfunction, DNA damage, lipid peroxidation, and protein oxidation. This oxidative stress has been implicated in the development and progression of various diseases, including cardiovascular diseases, neurodegenerative disorders, cancer, and inflammatory conditions. Antioxidants help to maintain the balance between ROS production and elimination, protecting cells from oxidative damage and promoting overall health.

In Vitro Assays for Assessing Antioxidant Property:

DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay:

- Measures the ability of antioxidants to scavenge the stable radical DPPH
- Changes in color indicate the reduction of DPPH, indicating antioxidant activity
- Spectrophotometric or colorimetric measurement of absorbance is used to quantify the antioxidant capacity

ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) Assay:

- Measures the ability of antioxidants to scavenge the ABTS radical cation
- Changes in color indicate the reduction of ABTS, indicating antioxidant activity
- Spectrophotometric or colorimetric measurement of absorbance is used to quantify the antioxidant capacity

FRAP (Ferric Reducing Antioxidant Power) Assay:

- Measures the reduction of ferric ions (Fe3+) to ferrous ions (Fe2+) by antioxidants

- Changes in color indicate the reduction of ferric ions, indicating antioxidant activity
- Spectrophotometric or colorimetric measurement of absorbance is used to quantify the antioxidant capacity

Total Phenolic Content Assay:

- Measures the total phenolic content in a sample, as phenolic compounds are known antioxidants
- Utilizes colorimetric or spectrophotometric methods using specific reagents
- Quantification is typically based on the absorbance measurement at specific wavelengths

Significance of In Vitro Findings:

In vitro studies provide valuable insights into the antioxidant potential of substances, allowing researchers to screen and compare different compounds for their ability to scavenge free radicals and protect against oxidative stress. These studies serve as a preliminary step in antioxidant research, helping to identify promising candidates for further evaluation in animal models and human clinical trials. In vitro findings can contribute to the understanding of the mechanisms of action of antioxidants, their bioavailability, and potential interactions with cellular components. Additionally, in vitro studies facilitate structure-activity relationship analyses, enabling the modification and development of novel antioxidant compounds with enhanced efficacy.

Limitations and Considerations:

It is important to acknowledge the limitations of in vitro studies for assessing antioxidant activity. In vitro assays may not fully replicate the complex cellular environment found in living organisms, and the relevance of in vitro findings to in vivo outcomes should be carefully interpreted. Factors such as absorption, distribution, metabolism, and excretion (ADME) properties may influence the bioavailability and effectiveness of antioxidants in the human body. Therefore, in vitro studies should be complemented with in vivo and clinical studies to validate the antioxidant potential and evaluate the overall health benefits.

RESULT

1. Percentage yield of extracts :

The extraction of Darjeeling tea and Filter Coffee was done using the Decoction method resulted in different percentage yields. The Tea exhibited percentage yield at 3.2%, and coffee exhibited percentage yield 5.66%

$$\textbf{Percentage Yield} = \frac{\textit{Weight of Extract}}{\textit{Weight of plant material}} \times 100$$

Table : Percentage yield of Darjeeling tea and Filter coffee

Plant	Weight of plant material (g)	Weight of extract (g)	Percentage yield (%)
Darjeeling tea	50	1.6	3.2
Filter coffee	50	2.83	5.66

2. Phytochemical analysis of extracts :

The phytochemical screening of the Darjeeling tea and Filter coffee revealed the presence of various bioactive compounds. The Darjeeling tea and Filter Coffee exhibited a diverse range of phytochemicals, including carbohydrates, flavonoids, tannins, phenolic compounds, glycosides and proteins

The presence of carbohydrates in both suggests the presence of sugars and polysaccharides, which can contribute to the potential therapeutic properties of the extracts. Carbohydrates are known to possess antioxidant, anti-inflammatory, and immunomodulatory activities.

The detection of flavonoids, tannins, and phenolic compounds in both is noteworthy. These phytochemicals are well-known for their antioxidant and anti-inflammatory properties. Flavonoids, in particular, have been extensively studied for their potential health benefits, including their ability to scavenge free radicals and modulate inflammatory pathways.

Table : Phytochemical screening of Darjeeling tea and Filter coffee

Phyto-constituent	Tests performed	Darjeeling tea	Filter coffee
Carbohydrates	Molisch's test	+	+
	Fehling's test	+	+
	Benedict's test	+	+

Proteins	Millon's test	+	+
	Biurate test	+	+
Amino acids	Ninhydrin test	+	+
Glycosides	Brontrager's test	+	+
	Baljet's test	+	+
Flavonoids	Shinoda test	+	+
	Lead acetate test	+	+
Tannins and phenolic compounds	a) 5% $FeCl_3$ solution test	+	+
	b) Lead acetate solution test	+	+
Alkaloids	a) Dragendorff's test	-	-
	b) Mayer's test	-	-
	c) Wagner's test	-	-
	d) Hager's test	-	-

3. Total Phenolic content

Comparing the TPC values, it is observed that the TPC of filter coffee is generally higher than that of Darjeeling tea at all three concentrations. At the lowest concentration (5µg/ml), the TPC of both Darjeeling tea and filter coffee is relatively close (6.4 and 6.6, respectively), indicating a similar phenolic content. However, as the concentration increases, the TPC of filter coffee shows a more significant increase compared to Darjeeling tea.

The TPC of both Darjeeling tea and filter coffee increases as the concentration of the sample increases. This suggests that higher concentrations of the samples allow for the extraction of a greater amount of phenolic compounds. The highest TPC values are observed at the highest concentration tested (15µg/ml), with 8.5 for Darjeeling tea and 11.5 for filter coffee

Phenolic compounds are known for their antioxidant properties. Higher TPC values indicate a higher concentration of phenolic antioxidants in the tea or coffee samples. Therefore, the higher TPC observed in filter coffee suggests that it may possess stronger antioxidant activity compared to Darjeeling tea.

Table : Total Phenolic content of Darjeeling tea and Filter coffee

SAMPLE	CONCENTRATION (µg/ml)	ABSORBANCE	AAE/g
Darjeeling Tea	5	0.093	6.4
	10	0.098	6.7
	15	0.130	8.5
Filter coffee	5	0.097	6.6
	10	0.110	7.3
	15	0.181	11.5

4. Phosphomolybdate assay

The phosphomolybdenum reagent assay is a commonly used method to evaluate the antioxidant activity of plant extracts or compounds. In this study, the assay was performed to assess the antioxidant potential of the Darjeeling tea and Filter coffee using ascorbic acid as the standard antioxidant.

The results revealed that the Filter Coffee exhibited a significant antioxidant activity with a percentage antioxidant activity of 82.1%. This indicates that the Filter coffee possesses strong antioxidant properties, as it demonstrated a high ability to scavenge free radicals and reduce oxidative stress. The high antioxidant activity of the Filter coffee suggests the presence of bioactive compounds with potent antioxidant effects.

The Darjeeling tea also showed notable antioxidant activity, albeit to a lesser extent compared to the Filter coffee. The percentage antioxidant activity of the Darjeeling tea was found to be 76.1%. This suggests that the Darjeeling tea contains compounds that can effectively neutralize free radicals and provide protection against oxidative damage.

(%) Percentage Antioxidant activity

$$\frac{Absorbanc(control) - Absorbance(sample)}{Absorbance(control)} \times 100$$

Table 1.6 : Percentage antioxidant activity of Darjeeling tea and Filter coffee

Sample	Absorbance	Percentage Antioxidant activity (%)
Ascorbic acid	2.948	---
Darjeeling tea	0.679	76.1
Filter coffee	0.526	82.1

5. Hydrogen Peroxide scavenging assay

The hydrogen peroxide scavenging activity assay measures the ability of a substance to neutralize hydrogen peroxide, a reactive oxygen species that can cause cellular damage. In this case, the hydrogen peroxide scavenging activity of Darjeeling tea and filter coffee was evaluated, with ascorbic acid serving as the standard for comparison. Let's discuss the findings:

The results indicate that both Darjeeling tea and filter coffee possess significant hydrogen peroxide scavenging activity. The percentage inhibition of hydrogen peroxide by Darjeeling tea was found to be 75.3%, while filter coffee exhibited a slightly higher percentage inhibition of 82.9%.

The hydrogen peroxide scavenging activity is a measure of the antioxidant potential of a substance. A higher percentage inhibition indicates a greater ability to neutralize hydrogen peroxide and protect against oxidative stress. The results suggest that both Darjeeling tea and filter coffee have strong antioxidant properties, as they were able to inhibit hydrogen peroxide to a considerable extent.

While both Darjeeling tea and filter coffee showed significant hydrogen peroxide scavenging activity, filter coffee exhibited a slightly higher percentage inhibition. This may be attributed to the differences in the chemical composition and the presence of specific antioxidants in coffee that contribute to its higher scavenging activity.

Percentage antioxidant activity(%)= $\frac{Absorbance(control)-Absorbance(sample)}{Absorbance(control)}$x100

Table : Hydrogen peroxide scavenging activity of Darjeeling tea and Filter coffee

Sample	Absorbance	Percentage antioxidant activity(%)
Ascorbic acid	1.154	---
Darjeeling Tea	0.285	75.3
Filter coffee	0.197	82.9

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