Comparative Study of Antioxidant Property of Coffee and Its Additives

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Authors’ contributions

This work was carried out in collaboration between both authors. Author DB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SB and DB managed the analyses of the study. Author SB managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Coffee, one of the most commonly consumed beverages is a very rich source of antioxidants alongside various other health benefits. The roasted beans of coffee are the seed of berry obtained from coffea species. These roasted coffee beans are utilized to prepare coffee. India is today producer of 16 unique varieties of coffee most of which originate from southern India. The goodness of coffee and Ganoderma can do wonders to human health. This study is aimed to have an analysis of antioxidant properties of instant coffee, filter coffee, coffee with Ganoderma extracts and ginger coffee by estimating the Ascorbic Acid equivalents (AAE). The essence of the study is the presence of additions and the antioxidant activity of the coffee samples in their presence. The two infusions used in the study are extracts of Ganoderma and ginger. The study also aims to have an inter group analysis of antioxidant properties of all the samples. The study was conducted using basic colorimetric techniques.

Keywords: Antioxidants; Ascorbic Acid Equivalents (AAE); coffee; Ganoderma; ascorbic; radical.
1. INTRODUCTION

The roasted beans of coffee are the seed of berry obtained from coffea species. These roasted coffee beans are utilized to prepare coffee by hot water extraction. It is a dark coloured, vicious, somewhat acidic beverage that has exciting effects in human, mainly because of its caffeinest contents. India is today producer of 16 unique varieties of coffee most of which originate from southern India. The two major species of coffee plants used for extraction of the produce are Coffea canephora and Coffea arabica [1], which are commonly known as "robusta" and "arabica" respectively. Arabica contributes to over 40-60% of the production whereas robusta does it over 20-40%. Arabica is more prevalent as it is less bitter and has more flavour when compared to the other variant. On the other hand, Robusta is known to have more caffeine content and a better body than arabica which explains its presence in various coffee blends. The HPLC analysis of coffee shows the presence of phenolic compounds like chlorogenic acids, caffeic acids, nonphenolic compounds caffeine, trigonellines, nicotinic acids and 5- (hydroxymethyl) furfuraldehydes continued in roasted coffee residue [2]. Coffee’s health benefits account mainly from its antioxidants. Most of the constituents of coffee act as antioxidants and are proven to reduce the risk of occurrence of many diseases and cell damage [3] by scavenging free radicals.

1.1 Free Radicals

So called free radicals are known/defined as, "the molecule species which are proficient of existing independently and consist of unpaired electrons in the atomic orbitals as well as captures electron from several other substance to get into the neutral state" [4]. Free radicals are synthesized in the body due to various metabolic activities, enzymatic and non-enzymatic reactions. Free radical are formed as a product of vital metabolic activities in mitochondria’s, peroxisomes and phagocytotic cells. They are also produced inside the body due to consumption of alcohol, tobacco, and few drug like halothanes and paracetamols and also as a result of exposure to pollution, heavy metal, transition metal, industrial solvent, pesticide and radiation [5].

The most important reactive oxygen species in numerous illness conditions are hydroxyl radicals, superoxide anion radicals, hypochlorite, nitric oxide radical hydrogen peroxides, oxygen singlets and peroxynitrite radical. The role of ROS and RNS can be substantiated as bi-phased because these reactive oxygen or nitrogen species in low concentration are said to be involved in various physiological mechanisms involved in the body and the higher concentration or accumulation of these radicals lead to deleterious effects. As the free radicals formed in the body are characterized by unpaired electrons, they attack vital cell components like proteins, lipids and nucleic acids in order to stabilize themselves, which lead to chain initiation and propagation. The first free radicals’ twists electrons from molecules that threatens the structure of molecules and turn them into the free radicals. Initiating a chain reaction, these molecules then take electrons from other molecules, threatening their structural integrity and turning them into new free radicals. This domino effect can ultimately disturb and harm the entire cell. The termination of free radicals occurs only when it is either scavenged by an antioxidant or is neutralised by reacting with other free radical or cell component [6]. These reactions lead to severe cell damage which lead to ageing and catastrophic diseases like cancer, cardiovascular disease, etc. Compounds capable of quenching or scavenging free radicals are called antioxidants.

1.2 Antioxidants

Antioxidant is a termed given to a group of several distinct chemical compounds that have the property to fight the effects of free radicals that are highly reactive and harmful for human body. These free radicals are formed several oxidative reactions that starts by the ingestion of the food or simply breathing. These antioxidants are defined as the substances at low concentration which suppress the oxidation of different substrates. The human body has a mechanism through which it synthesizes antioxidants inside the body in order to curb the deleterious effects of free radicals. Lifestyle changes in today’s world (consumption of tobacco and fats, smoking) and effects of various pesticides have led to an increased production of free radicals in the body. In order to scavenge or quench these free radicals, exogenous source of antioxidants in the diet is very important [7].

2. REVIEW OF LITERATURE

Formation and accumulation of free radicals are known to cause disastrous effects in the human body as they oxidise the vital cell components in
order to neutralise them. The naturally produced antioxidants are the ones that scavenge these free radicals resulting in prevention of the consequences [8].

The human body maintains a complex mechanism in order to maintain the equilibrium between the amount of free radical generated and the number of antioxidants produced. The failure of maintaining this equilibrium i.e. the accumulation of free radicals in the body leads to “oxidative stress” [9]. Oxidative stress is the major cause of occurrence of severe disastrous cell damage that may lead to diseases like cancer, cardiovascular disease and also the effects of ageing. Coffee apart being one of the majorly consumed beverages globally is also a potent source of antioxidants. The phenolic and the non-phenolic counterparts of bioactive component profile of coffee are proven to possess the antioxidant activity [2]. coffee is one of the most consumed exogenous sources of antioxidants along with fruits, wine and dark chocolate [10].

The antioxidant activity of coffee and additions namely Ganoderma and ginger are proven to be of a significant contributor when consumed individually [11,12]. The antioxidant activity of coffee can further be estimated in the presence of these infusions to check the effect of these infusions when combined with coffee. The study can further be elaborated by characterizing the kind of bioactive component responsible for this activity.

3. METHODOLOGY

3.1 Design

The principle behind the Folin Ciocalteau’s Reagent Method (FCR) method (Fig. 1) is the fact that, phenols present in the sample reacts with the oxidising agents phosphomolybdate in FC reagents in alkaline condition and leads in formations of complex of blue colour, the molybdenum blue that is calculated at 680 nm. The standard graph was prepared using 1 mg/mL of ascorbic acid as a standard and 4 aliquots of concentration ranging from 0.1 mg/ml to 0.025 mg/ml were used along with the blank. Distilled water was then added to all the tubes to make the total volume of 2 mL. After boiling the required concentration of coffee in 100 mL of distilled water for 5 minutes, the samples are taken for assay. The aliquots or concentrations of coffee samples used are 1.25 mg/mL, 0.625 mg/mL and 0.31 mg/mL. The volume was made to 2 mL using distilled water. After addition of water, 2.5 mL of FC reagent was added to all the tubes followed by incubation for 5 minutes. Upon incubation, 2 mL of Na₂CO₃ was added to all the tubes. The tubes were then incubated for 30 minutes at room temperature. The absorbance was measured at 680nm for both diluted and undiluted forms.

![Flowchart](image)

**Fig. 1. Flowchart representing the protocol followed to carry out the research study**
3.2 Samples

For this study, four commercially available coffee samples were selected and obtained from a grocery store. They were selected in order to estimate and compare the Ascorbic acid equivalents present in each of the coffee samples. Another aim of this selection was to compare their antioxidant activity with respect to the presence or absence of infusions or additives. Coffee with Ganoderma and ginger coffee were selected due to the presence of Ganoderma and ginger in them respectively. The physio-chemical study of all the samples was done which is discussed briefly in the results.

3.3 Instruments

FC reagent, Na$_2$CO$_3$ (sodium carbonate) and Ascorbic acid. The method was standardised according to the lab and the equipment used. The concentrations of the stock coffee samples were checked according to the colorimeter as the colour of the coffee and the colour of the FC reagent would give a very high value. The volume of the reagents namely FC reagent and Na$_2$CO$_3$ were modified and set according to the range of the colorimeter. The incubation time was also set by checking the durations of 30 minutes and 60 minutes. Current research was conducted by following the FCR. This method is used to calculate the ascorbic acid equivalents (AAE) present in the sample.

3.4 Data Collection

3.4.1 Physico-chemical evaluation of samples as shown in Fig. 2

- Instant coffee
  - Colour-dark brown
  - Texture- uniform fine powder
  - Solubility- easily soluble in water
- Coffee with Ganoderma extract
  - Colour- lighter than instant coffee
- Ginger coffee
  - Colour- light brown
  - Texture- coarse coffee particles
  - Solubility- not easily soluble in water and requires a bit of grinding
- Filter coffee
  - Colour- dark brown
  - Form: homogeneous solution of coffee decoction

3.4.2 Preparation of standard graph

A standard graph was plotted for each of the sample, based on the OD values obtained. A representative of the standard graph is shown in Fig. 3.

The OD values of all the samples in their respective concentration is as follows in Table 1.

A representative image of one of the samples used during the assay is given below in Fig. 4.

| Coffee samples                        | OD at 0.625mg/mL | OD at 0.31mg/mL |
|---------------------------------------|------------------|-----------------|
| Instant coffee                        | 0.58             | 0.26            |
| Coffee with Ganoderma extract         | 0.65             | 0.31            |
| Ginger coffee                         | 0.04             | 0.02            |
| Filter coffee                         | 0.34             | 0.18            |
3.4.3 Calculation of ascorbic acid equivalents

The calculation of Ascorbic Acid Equivalents (AAE) is done using the formula \( y = mx + c \),

where, \( y = \) OD value obtained, \( m = \) slope of the graph (standard).

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On obtaining the value of \( x \), the Ascorbic acid Equivalents are calculated for 1g of the sample.

The values of Ascorbic acid equivalents at 0.625 mg/mL after calculation using the above-mentioned formula are in Table 2.

The values of Ascorbic acid equivalents at 0.31 mg/mL after calculation using the above-mentioned formula are in Table 3.

### 3.5 Data Analysis

The calculation of Ascorbic Acid Equivalents (AAE) is done using the formula “\( y = mx + c \)”, where, \( y = \) OD value obtained, \( m = \) slope of the graph (standard). It was reported that the value of Ascorbic acid equivalents at 0.625 mg/ml for instant coffee was 88 \( \mu \)g, for coffee with Ganoderma AAE was 100 \( \mu \)g, for ginger coffee AAE was 8 \( \mu \)g and for filter coffee AAE was 45 \( \mu \)g as shown in Fig. 5. Whereas at 031 mg/ml AAE for instant coffee was 80 \( \mu \)g, for coffee with Ganoderma AAE was 96 \( \mu \)g, for ginger coffee AAE was 8.64 \( \mu \)g and for filter coffee AAE was 46 \( \mu \)g as shown in Fig. 6.

#### Table 2. Ascorbic acid equivalents (AAE) at 0.625 mg/mL

| Coffee samples          | AAE (\( \mu \)g) |
|-------------------------|------------------|
| Instant coffee          | 88               |
| Coffee with Ganoderma   | 100              |
| Ginger coffee           | 8                |
| Filter coffee           | 45               |
Table 3. Ascorbic acid equivalents (AAE) at 0.31 mg/mL

| Coffee samples       | AAE (µg) |
|----------------------|----------|
| Instant coffee       | 80       |
| Coffee with Ganoderma| 96       |
| Ginger coffee        | 8.64     |
| Filter coffee        | 46       |

Fig. 5. Comparative analysis of antioxidant activity based on ascorbic acid equivalents

Fig. 6. Comparative analysis of antioxidant activity based on ascorbic acid equivalents

4. RESULTS AND DISCUSSION

The inter group comparison of coffee samples using instant coffee as the reference shows the fold increase or decrease in the antioxidation activity in term of Ascorbic Acid equivalent. Instant coffee is taken as a reference because majority of the samples are an instant coffee formulation and it is also a coffee without the presence of infusions. Coffee with Ganoderma extracts shows to have a slightly greater number of Ascorbic acid equivalents as compared to the reference. The values obtained after the calculation of fold change are shown in the Fig. 7.
5. CONCLUSION

Among the different sources of antioxidants, coffee is proven to be one of the major sources of antioxidants in comparison to various other commonly consumed ones like cocoa, dark chocolate, wine and fruits/vegetables. Apart from being a rich source of antioxidants and also one of the most consumed beverages globally, coffee is also known to contain various micronutrients like vitamin B2, vitamin B3 etc. The antioxidant property of coffee attributes to its free radical scavenging activity. Coffee constitutes of both phenolic and non-phenolic compounds which may be responsible for its antioxidant properties. Phenolic compounds like chlorogenic acid and caffeic acid and non-phenolic compounds like caffeine can be considered as the bioactive component. Free radicals produced in the body are important for physiological functions like apoptosis of cells, but when accumulated can cause damage to cell components like proteins and nucleic acids and can thereby lead to severe conditions like cancer. The human body ideally maintains an equilibrium between the free radicals produced and the naturally produced antioxidants. Any change or disturbance in this equilibrium due to various lifestyle activities like smoking and effects of pesticides and radiation can lead to Oxidative stress. Exogenous sources of antioxidants like coffee come into picture in order to scavenge the free radicals by reducing and neutralizing them and thereby reducing the oxidative stress. The study briefly describes the antioxidant activity of any compound that has a ‘polyphenol’ structure present in all the coffee samples. The antioxidant’s property is estimated in term of Ascorbic Acid Equivalent using Ascorbic acid as the standard.

In this study, ascorbic acid is used as a standard for calculation of Ascorbic acid equivalents due to its reducing capacity and its presence in human body. Various other standards like gallic acid can also be used. In our scenario, ascorbic acid is preferred due to its availability and economic feasibility. The results of filter coffee are satisfactory with respect to the amount of Ascorbic acid equivalents estimated. The coffee to water ratio and also the extraction time plays a vital role in detaining the antioxidants in the brew. These factors vary significantly from sample to sample and can be estimated only with respect to the sample acquired. The further antioxidant profile of all the coffee samples can be checked by following various protocols that estimate the total antioxidant activity of the same. The total antioxidant activity estimates phenolic compounds that are potential antioxidants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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