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**Research Article** 

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## In vitro Antioxidant Analysis of Selected Coffee Bean Varieties

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## ABSTRACT

Coffee is a brewed beverage prepared from the roasted seeds of the coffee plant. It is prepared from the roasted beans (seeds) of coffee plant. It is one of the most-consumed beverages in the world. It was believed that coffee consumption was not good for health. But now a days many reseachers have reported various health benefits of coffee consumption. The aim of the present investigation was to carry out the priliminary phytochemical analysis and in vitro antioxidant analysis of selected coffee bean varieties. Two varieties of Arabica type namely "Special A" and "Kumbakonam" was selected for the present study. Fresh coffee brews were prepared from both the varieties and used for following analysis. Preliminary phytochemical analysis was done by standard methods. Quantitative analysis like estimation of total phenols and flavonoids were also done on both the varieties. Radical scavenging assays like DPPH Radical Scavenging assay, Nitric oxide Radical scavenging assay, Hydroxy Radical scavenging assay, Superoxide Radical scavenging assay and Total Reducing power assay was also done on both the SpecialA and Kumbakonam varieties. The result showed that Kumbakonam variety has greater phenol and flavonoid content than SpecialA.DPPH, Nitric oxide and Hydroxy radical scavenging assay showed that Special A variety was an excellent scavenger of these radicals especially Nitric oxide radical (P < 0.05). Superoxide Radical scavenging assay and Total Reducing power assay results depicts that kumbakonam variety was a good superoxide radical scavenger (P < 0.05). Thus, it was concluded that coffee was rich in antioxidants which could help the mankind to combat various ailments which involves oxidative stress in their pathogenesis.

Keywords: Coffee, Antioxidants, DPPH, Arabica, beverage.

## INTRODUCTION

Reactive oxygen species (ROS) have been recognized as playing an important role in the initiation and/or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular disease. Reactive oxygen species (ROS) are generated by normal metabolic processes in all oxygen utilizing organisms. It is estimated that about 1% of the total oxygen consumed by mitochondria is transformed into superoxide anion[1]. About 95% of the pathologies observed in people above 35 years of age are associated with production and accumulation of free radicals [2]. Herbal medicine or phytomedicine refers to the use of any plant seeds, berries, roots, leaves, bark or flowers for medicinal purposes. Plant drugs could be effective and at the same time have less or no side effect. Now a days 80% people (WHO estimated) from all over world are interested towards traditional medicines [3].

Antioxidant properties elicited by plant species have a full range of perspective applications in human healthcare. It is widely accepted that a plant-based diet with high in take of fruits, vegetables and other nutrient - rich plant foods may reduce the risk of oxidative stress -related diseases [4-9]. It is hypothesized that antioxidants originating from foods may work as antioxidants in their own right *in vivo*, as well as bring about beneficial health effects through other mechanisms, including acting as inducers of mechanisms related to antioxidant defense [10,11], longevity

[12,13], cell maintenance and DNA repair [14]. In search for sources of novel antioxidants, in the last few years some medicinal plants have been extensively studied for their radical scavenging activity[15].

Coffee is one of the most popular beverages consumed daily throughout the world. Coffee is a brewedbeverage with a dark, acidic flavor prepared from the roasted seeds of the coffee plant. Coffee plants are classified in the large family Rubiaceae. They are evergreen shrubs or small trees that may grow 5 m (15 ft) tall when unpruned. The leaves are dark green and glossy, usually 10–15 cm (4–6 in) long and 6 cm (2.4 in) wide. The flowers are axillary, and clusters of fragrant white flowers bloom simultaneously and are followed by oval berries of about 1.5 cm [16]. Green when immature, they ripen to yellow, then crimson, before turning black on drying. Each berry usually contains two seeds, but 5–10% of the berries have only one; these are called peaberries [17]. Berries ripen in seven to nine months. According to Svilaas, coffee based drinks contribute to 64% of the total antioxidant intake, followed by fruits, berries, tea, wines, cereals and vegetables [18]. Various studies suggests that coffee consumption reduces the risk of being affected by Alzheimer's disease, Parkinson's disease, heart disease, diabetes mellitus type 2, cirrhosis of the liver, cancer [19] and gout.

Of the two main species grown, arabica coffee (from C. arabica) is generally more highly regarded than robusta coffee (from C. canephora); robusta tends to be bitter and have less flavor than arabica. The present study was aimed at to perform the Preliminary Phytochemical analysis(Qualitative and Quantitative Analysis)and*In vitro* antioxidant assessment of selected local coffee bean(Arabica)varieties namely, Special-A and Kumbakonam.

## EXPERIMENTAL SECTION

## Chemicals

Catechin,Curcumin, quercetin, gallic acid,1,1-diphenyl phenyl hydrazyl(DPPH) were purchased from Sigma, St.Louis, MO, USA. All other reagents used , including solvents were of analytical grade and obtained from Himedia, India. Recordings were made in a UV-Vis spectrometer (Shimadzu UV-2200).

## **Sample Collection**

Roasted Coffee beans (Arabica) of two local varieties namely "Special A" and "Kumbakonam" were purchased from Vivekananda Coffee shop, Adyar, Chennai.

## **Coffee Brew Preparation**

The coffee beans were finely ground and the brew was prepared by solid-liquid extraction with deionized water. The coffee powder (1 g) was mixed with 100 ml deionised water at 90°C and extracted for 10 minutes. The samples were then filtered through Whatman No. 1 filter paper. All analyses were performed with freshly prepared coffee brews.

## **Preliminary Phytochemical Analysis**

Qualitative analysis was done by the method of Sofowara, 1993 [20], Trease and Evans, 1989 [21] and Harborne,1973 [22].Total phenols was estimated by the Folin- ciocalteau(1927) method[23]. Total flavonoids was estimated by the method of Chang C et al ,2002 [24].

## In vitro anti oxidant analysis

The DPPH radical scavenging activity of CoffeeBrew was measured by the method of Blois MS,1958 [25].Nitric oxide radical scavenging activity of CoffeeBrew was measured based on Griess reaction[26].The superoxide anion scavenging activity of CoffeeBrew was determined by the method described by Nishimiki *et al.*,(1972) slightly modified[27]. The hydroxyl radical scavenging activity was determined according to the method reported by Klein and co- workers,1992 [28].The total reducing power ofcoffeeBrew was determined by the method of Oyaizu M,1986[29].All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

## **Statistical Significance**

The values were expressed as mean  $\pm$  SD. Statistical analysis was done by student't' test and 'P' value was arrived at to access the statistical significance of changes observed. P value less than 0.05 was considered as significant.

## **RESULTS AND DISCUSSION**

Table 1 shows the presence of tannins, alkaloids, flavonoids, carbohydrates, quinones, phenols, coumarin, starch & fixed oil and shows the absence of steroids, cardiac glycosides, saponins, proteins & gum in coffee brew.

PHYTOCHEMICALS	SPECIAL A	KUMBAKONAM
Tannins	+	_
Saponins	_	_
Cardiac glycosides	_	_
Steroid	_	_
Alkaloids	+	+
Flavonoids	+	+
Carbohydrates	+	+
Protein	_	_
Coumarin	+	+
Quinones	+	+
Phenols	+	+
Gum	_	_
Starch	+	+
Fixed oil	+	+

## Table 1. Qualitative Analysis of Phytochemicals in Coffee Brew

## Table 2 : Total Phenol and Flavonoid Levels of Selected Coffee Bean Varieties

QUANTITATIVE ANALYSIS	SPECIAL-A	KUMBAKONAM
TOTAL PHENOLS (mg of GAE/serving)	0.03	0.06
TOTAL FLAVONOIDS (mg of CE /serving)	0.02	0.03

Table 2 depicts the levels of total phenols and flavonoids in Special A and Kumbakonam varieties. Among these two coffee bean varieties, Kumbakonam was found to contain much phytochemicals than Special A beans. These phenols and flavonoids can donate their extra hydrogens to reduce various free radical and thereby they exert the antioxidant activity. Thus these can act as singlet oxygen quenchers, metal chelators.

## IN VITRO antioxidant activity:

The potentially reactive derivatives of oxygen ascribed as ROS such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide are continously generated inside the human body as a consequence of exposure to exogenous chemical and/or a number of endogenous metabolic processes involving redox enzymes and bioenergetic electron transfer [30]. Owing to the ROS overproduction and/or inadequate antioxidant defense, there is upsurge of ROS and this culminates in oxidative stress. It is quite interesting to note that plant have good antioxidant ability and are safer than the synthetic antioxidants. The antioxidant activity can be attributed to various mechanism like prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, reductive capacity, and radical scavenging activity. In the present study, five different antioxidant methods for evaluation of antioxidant activity have been used.

Several concentrations ranging from 20 - 100  $\mu$ g/mL of coffee brew were tested for their antioxidant activity using different *in vitro* models. It was observed that free radicals were scavenged by the test compounds in a dose dependent manner in the various methods.

## DPPH radical scavenging activity.

DPPH is a free radical, stable at room temperature, which produces a violet solution in ethanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncolored ethanol solutions. The use of DPPH provides an easy and rapid way to evaluate antioxidant activity. The DPPH assay is mainly based on an electron transfer reaction, and hydrogen atom transfer reaction is a marginal reaction pathway. However, DPPH colour can be lost via unrelated reactions, and steric accessibility is a major determinant of the reaction [31, 32]. Furthermore, the diversity and complexity of the extracts increase the complication of mechanism of DPPH assay. In this system, the structure (both planar and spatial) of the antioxidant compound, present in the extract, is important for its capacity of donating hydrogen ions. Compounds able to donate hydrogen are derived from the shikimate pathway, as for example, flavonoids [33].

The DPPH radical is considered to be a model of lipophilic radical. A chain reaction in lipophilic radicals was initiated by lipid autoxidation. The radical scavenging activity of coffee brew was determined from the reduction in absorbance at 517 nm due to scavenging of stable DPPH free radical. The positive DPPH test suggests that the samples are free radical scavengers. The scavenging effects of Special A and Kumbakonam on the DPPH radical are illustrated and compared in **Figure 1**. Coffee brew had significant scavenging effects on the DPPH radical which increased with increasing concentration in the 20 - 100  $\mu$ g/ml range;kumbakonam variety had high DPPH radical

compare to Special-A. the scavenging effect of coffee brew was similar to that of BHA. The IC<sub>50</sub> value of coffee brew is 96 $\mu$ g/ml in sepecial-A ,90 $\mu$ g/ml in kumbakonam and BHA in the DPPH radical scavenging assay was 38.5  $\mu$ g/ml, a statistically significant result (P < 0.05).





## Nitric oxide radical scavenging activity

NO is an important chemical mediator generated by endothelial cells, macrophages, neurons and is involved in the regulation of various physiological processes [34]. Excess concentration of NO is associated with several diseases [35,36].Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions which acts as free radicals [37]. Nitric oxide can react rapidly in the intracellular environment to form nitrate, nitrite and s-nitrosothiols. These metabolites play a key role in mediating many xenotoxic effects such as DNA damage. NO causes DNA damage via peroxynitrite.



Figure 2. Nitric oxide radical scavenging activity

In the present study, the Coffee Brew of two selected coffee beans were checked for its inhibitory effect on nitric oxide production. Figure 2 illustrates the percent inhibition of nitric oxide generation by Coffee Brew of two selected coffee beans. Curcumin was used as a reference compound. The concentration of Coffee Brew needed for 50% inhibition was 12µg/ml in Special-A , 16 µg/ml in Kumbakonam, whereas 20.4 µg/ml was needed for an equal weight of curcumin. The results were statistically significant (P < 0.05).

#### Superoxide anion radical scavenging activity

Superoxide anion is produced from molecular oxygen due to oxidative enzymes [38] of body by non enzymatic reaction such as autooxidation by catecholamine [39]. The scavenging activity towards the superoxide radical is measured in terms of inhibition of generation of  $O_2^-$ . In the present study superoxide radical reduces NBT to blue coloured formazan that is measured at 560 nm [40].

Superoxide anions indirectly initiate lipid oxidation as a result of superoxide and hydrogen peroxide, serving as precursors of singlet oxygen and hydroxyl radicals [41]. Robak and Glyglewski [42] reported that the antioxidant properties of flavonoids are effective mainly via the scavenging of superoxide anion. The results are given in Figure 3. The IC<sub>50</sub> value of Kumbakonam and Special A on superoxide radical scavenging activity was found to be 85  $\mu$ g/ml A & 80  $\mu$ g /ml, whereas the IC<sub>50</sub> value of BHT and quercetin was 22.77 and 31.58  $\mu$ g/ml, respectively. The results were statistically significant (P < 0.05).



## Figure 3. Superoxide anion radical scavenging activity

Figure 4. Hydroxyl radical scavenging activity



## HYDROXY RADICAL SCAVENGING ACTIVITY:

Hydroxyl radical is an extremely reactive species formed in biological systems and has been implicated as highly damaging in free radical pathology, capable of damaging almost every molecule foundin living cells [43]. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis,

## Gayathri Gunalan et al

mutagenesis and cytotoxicity. In addition, this species is considered to be one of the quick initiators of the lipid per oxidation process, abstracting hydrogen atoms from unsaturated fatty acids. Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid-iron EDTA. The hydroxyl radicals formed by the oxidation react with DMSO to yield formaldehyde, which provides a convenient method for their detection by treatment with Nash regent.

In the present study, the coffee brew of two selected coffee beans were checked for its inhibitory effect on Hydroxyl radical production. Figure 4 illustrates the percent inhibition of Hydroxyl radical by coffee brew . BHT (43  $\mu$ g/ml) and Catechin (77.5  $\mu$ g/ml) was used as a reference compounds. The concentration of coffee brew needed for 50% inhibition was 136  $\mu$ g/ml in Special-A and 150 $\mu$ g/ml in Kumbakonam. The results were not significant (P < 0.05) as compared to reference compounds.

## TOTAL REDUCING POWER



Figure 5. Total reducing power assay

**Figure 5** shows the reducing capacity of coffee brew of two selected coffee beans were compared with BHT. Antioxidant activity has been reported to be concomitant with the development of reducing power [44]. Okuda *et al.*[41] reported that the reducing power of tannins prevents liver injury by inhibiting the formation of lipid peroxides. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [45].

#### CONCLUSION

Thus, the present study demonstrates that consumption of coffee may increase the total antioxidants level in plasma. Hence it can be concluded that coffee was also a good antioxidant besides green tea,cocoa,red wine etc. As a most consumed beverage,coffee can contribute much phytochemicals to man which would help him to boost his immunity and to fight against various diseases.

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