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## Antioxidant Capacity of a Novel Spice Refresher Drink

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### Abstract

A novel refresher drink made of a natural matrix that contains spice extracts of turmeric, ginger, black pepper and green tea was formulated. In addition to the spice component, this drink contained sucrose, citric acid and sodium chloride. The antioxidant activity of such a drink was determined by an oxygen radical absorbing capacity (ORAC) assay, utilizing a trolox equivalence method. A trolox standard curve was established. Determinations of the antioxidant capacity of this refresher drink involved five samples of 4 different levels used. Results showed trolox equivalency values of various concentration of refresher drink are 0,  $0.289 \pm 0.02$ ,  $0.564 \pm 0.05$  and  $0.827 \pm 0.06$   $\mu\text{g/ml}$  for the concentrations of 0, 125, 250 and 500  $\mu\text{g/ml}$ , respectively. It is proved that the antioxidant property of refresher drink is highly dose dependent. The high ORAC value of this drink indicates that it can be considered as an oxidative stress reliever, with favorable effects on human health.

**Keywords:** Turmeric; Ginger; Black pepper; Polyphenols; ORAC activity

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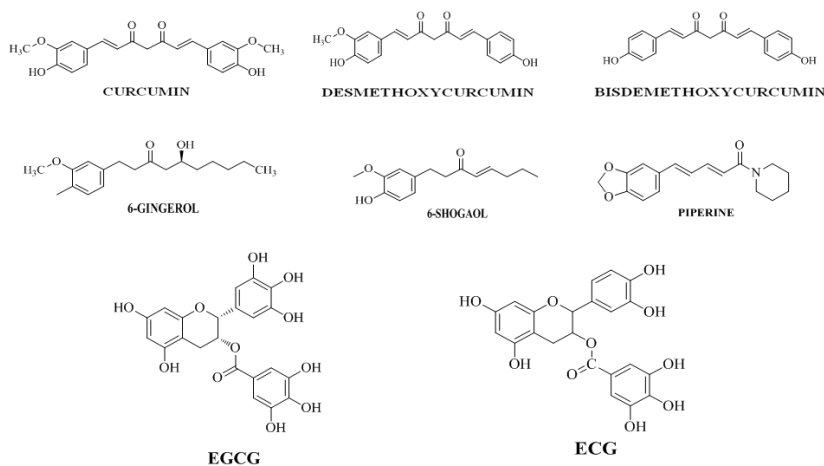
### Introduction

Consumption of refresher drinks is particularly prevalent in adolescents and young adults, with reports of more than 30% of this population consuming these drinks on a regular basis [1,2]. These young people are selectively targeted by refresher drink manufacturers, and with aggressive publicity campaigns, consumption of such drinks is increasing [3,4]. The use of plant based drinks like herbal teas, particularly green tea, and herbal powders are common and have no adverse side effects. Nowadays nearly 25% of all drugs or medicines in the developed world contain constituents derived from medicinal plants [5,6]. Recently, the knowledge of herbal prescriptions is used by chemists to manufacture different effective mixtures.

Plant derived phytochemicals possess potential ability to act as antioxidants by free radical or reactive oxygen species scavenging [7]. Flavonoids and other phenolic compounds in plant extracts are responsible for the exhibited potent antioxidant activity. One such plant with a pool of antioxidant compounds is turmeric (*Curcuma longa* Linn) [8]. The phytochemicals that are responsible for the antioxidant properties of turmeric include curcuminoids [9]. Chemical structures of curcuminoids include: curcumin, desmethoxy curcumin and bisdemethoxy curcumin (Figure 1). Curcumin exhibits antioxidant activity by inhibiting lipid peroxidation and oxidative DNA damage. Curcuminoids induce glutathione-S-transferase and inhibit the cytochrome P 450 [10]. Also, curcuminoids exhibit pro-oxidant behavior

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because of the special conjugated  $\beta$ -diketone structure [11]. The most important mechanism by which curcumin exhibits its antioxidative action is by donating an H-atom from the phenolic group. The hydrogen atom transfer mechanism is initiated by the formation of a delocalized stable radical that inhibits the propagation of chain reactions or slows down the rate of propagation. A chain breaking antioxidant (CBA) donates the labile hydrogen atom to active oxygen species. The carbon radical ( $C^\bullet$ ) from an antioxidant is stable and is not able to continue the propagation of auto oxidation of the chain [12].



**Figure 1:** Chemical structure of curcuminoids, 6-gingerol, 6-shogaol, piperine, EGCG and ECG.

Ginger is widely cultivated in subtropical regions, where it is used as a medicine and provides flavor in food preparations. It is one of the herbal plants used as spices in cooking and in medicinal remedies around the world. It is reported to have antioxidant, antimicrobial and anti-inflammatory activities. The quality attributes of ginger are mainly decided by the volatile essential oils and the non-volatile oleoresin [13]. These volatile oils include: zingiberene, curcumene and farnesene as major components [14], which determine the flavor note of the ginger. Also, these oils in ginger contain various sesquiterpenes [15]. The characteristic pungency of ginger is due to the non-volatile compounds, namely: 6-gingerol and shogaols (Figure 1) [16]. Ginger extract shows a role in scavenging superoxide anion and hydroxyl radicals and gingerol inhibited ascorbate/ferrous complex-induced lipid peroxidation in rat liver microsomes. Ginger and its constituents show antioxidant activity and prevent the damage of macromolecules that is caused by the free radicals/oxidative stress [17]. Ginger has been widely used for the preparation of refresher drinks most of the in Asian countries, including India.

Black Pepper (*Piper nigrum*) belongs to the Piperaceae family [18], which contains alkaloids as the important constituents of the pepper. Piperine (Figure 1) is one of the most important constituent; which exhibits antioxidative action by inhibiting lipid peroxidation, arresting different radicals like superoxides, free radicals, and other reactive oxygen species [19]. Tea, derived from *Camellia sinensis L.*, is one of the most widely consumed beverages in the world. The health benefits of green tea are well established. The catechin class of compounds, particularly epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) (Figure 1) are responsible for the potential health benefit of green tea. Polyphenols from green tea are functioning as antioxidants through inhibition of the redox-sensitive transcription factors, pro-oxidant enzymes, and induction of antioxidant enzymes such as glutathione-S-transferases and superoxide dismutases [20].

Antioxidants play a vital role in controlling healthy biological activities of the human body [21,22]. The oxygen consumption inherent in cell growth leads to the generation of a series of reactive oxygen species. Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. The class of ROS includes hydroxyl radical, superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and a class of lipid peroxides. All are capable of reacting with cell membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage that can eventually lead to cell death [23].

The term 'refresher drink' is defined as ready-to-consume beverage that is formulated with enhanced sensory properties like taste, color and smell. One or more bioactive ingredients are added to the formula for a certain health effect to those who consume it. These bioactive substances are usually isolated from plants, animals, and microbes - such as flavonoids, phenols, polysaccharides, oligosaccharides, special fatty acids, amino acids, phytoestrogen, polyphenols, and others. This study aimed to produce a refresher drink that contains a mixture of turmeric, ginger, black pepper and green tea. Another objective was to evaluate the antioxidant capacity of this novel spice drink formulae by utilizing an established oxygen radical absorbing capacity (ORAC) method. As this is the first formulation of a refresher drink containing a certain spice mixture, it was of interest to conduct such an evaluation.

## Materials and Methods

### Materials

Monosodium hydrogen phosphate and disodium hydrogen phosphate were purchased from Merck Chemicals, India. Trolox, fluorescein diacetate and 2, 2' Azobis (2-amidinopropane) dihydrochloride (AAPH) were purchased from Sigma Aldrich, India. A proprietary functional refresher drink (Oh!K) was prepared at our research and development laboratory; M/s Aurea Biolabs Pvt Ltd, Cochin, Kerala, India. All performed analyses utilized a Synergy HT Multi-Detection Microplate Reader (BioTek Instruments, USA).

### Formulation of the refresher drink

Ingredients used in the formulation of this drink were: water (80%), sucrose (18%), citric acid (1%), sodium chloride (0.5%) and a mixture of spice extracts (0.5%). The spice extract mixture contained turmeric (42%), ginger (28%), black pepper (17%), and green tea (13%) as bioactive ingredients. This refresher drink was formulated as a pure and natural matrix.

### Determination of antioxidant capacity

The antioxidant activity of the drink was determined using an oxygen radical absorbing capacity (ORAC) assay method described by Ou, *et al.* 2001, with some modifications [24]. This method provides a very unique and complete assessment in which the inhibition time and degree of inhibition are measured as the reaction goes to completion. The ORAC assay is based upon the inhibition of the peroxy radical-induced oxidation that is initiated by thermal decomposition of azo- compound, such as [2,2'-azobis (2-methylpropionamide) dihydro- chloride] (AAPH). The ORAC assay provides a direct measurement of hydrophilic chain breaking antioxidant capacity against the peroxy radical. Five replicates of the refresher drink were analyzed and the average values, mean and standard deviation are reported.

## Results and discussion

The spice extract mixture that included turmeric, ginger, black pepper, and green tea was a constituent of the formulated drink. The spice extracts were mixed with sucrose to protect the aroma compounds contained to interact with other ingredients and to help in remaining them to be stable. This formulation was subjected to ORAC assay. An ORAC value gives an indication of the antioxidant capacity of the product when compared to a trolox equivalent value (TE). Data of the obtained trolox standard curve, which was used to evaluate the ORAC score of the drink, are presented in Table 1. A graphical representation of the standard concentrations used to establish the standard curve is shown in Figure 2. The trolox values of the formulated drink in different concentrations are represented in Table 2. Results showed trolox equivalency values of various concentration of refresher drink are 0,  $0.289 \pm 0.02$ ,  $0.564 \pm 0.05$  and  $0.827 \pm 0.06$   $\mu\text{g/ml}$  for the concentrations of 0, 125, 250 and 500  $\mu\text{g/ml}$ , respectively. The reported ORAC values for ground turmeric, ginger, black pepper and green tea were found to be: 127068, 14840, 34053, 1253  $\mu\text{mol TE}/100\text{g}$  respectively [25].

The ORAC value of the formulated drink was determined to be 1,098,669  $\mu\text{mol TE}/100\text{g}$ ; it is very much higher value than the individual ground spices. Because of the formulated refresher drink has been prepared by the extracts of ground turmeric, ginger, black pepper and green tea. Moreover, the extracts naturally have high amount of ascorbic acid and citric acid, which are having high antioxidant property. The high ORAC value of refresher drink is proved that this functional drink has high antioxidant property. The antioxidant property of refresher drink is highly dose dependent; when the concentration increases the antioxidant property also increases (Table 2).

Trolox concentration	AUC	Net AUC
0 µg/ml	42.95 ± 0.05	0.00 ± 0.00
12.5 µg/ml	52.56 ± 0.07	9.61 ± 0.03
25 µg/ml	57.93 ± 0.02	14.98 ± 0.05
50 µg/ml	58.84 ± 0.12	15.89 ± 0.04
100 µg/ml	58.95 ± 0.09	16.00 ± 0.06
200 µg/ml	58.67 ± 0.04	15.72 ± 0.02

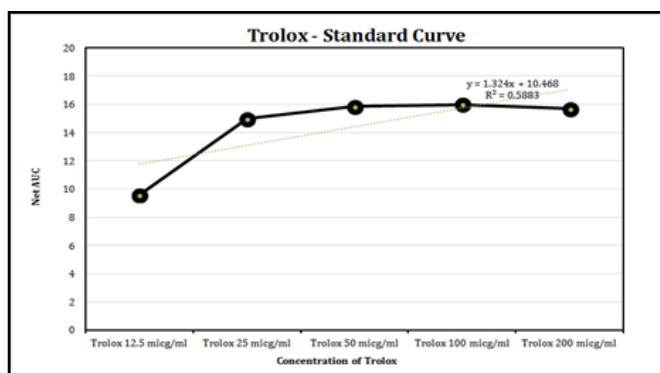
Values are means ± SD of five independent determinations

**Table 1:** Pharmacokinetic values of various concentrations of Trolox to plot a standard curve.

Concentration of drink (µg/ml)	AUC	Net AUC	Trolox equivalent (µg/ml)
0	44.33 ± 0.04	0.00 ± 0.02	-
125	44.71 ± 0.07	0.38 ± 0.04	0.289 ± 0.02
250	45.08 ± 0.03	0.75 ± 0.03	0.564 ± 0.05
500	45.43 ± 0.06	1.10 ± 0.05	0.827 ± 0.06

Values are means ± SD of five independent determinations

**Table 2:** ORAC Activity of tested items of the drink, expressed as trolox equivalents.



**Figure 2:** The established trolox standard curve.

Oxidative stress forced by ROS plays a crucial role in the pathophysiology associated with neoplasia, atherosclerosis and neurodegenerative diseases. The potential mechanism of the protective effects of phenolic compounds, including flavonoids, is thought to be due to their direct scavenging of free radicals [26]. The additive and synergistic effects of dietary phytochemicals obtained from fruits and vegetables are likely to be more effective in their anticancer activities than of a dietary supplement [27]. Nirmala., *et al.* [28] reported the in vivo antimutagenic effect of ginger in rats and suggested that the antimutagenic and chemopreventive potential of ginger could be due to its antioxidant activity. Barreira., *et al.* [29] studied the antioxidant activity of an herbal beverage including a formulation of *Camellia sinensis*, *Aspalathus linearis* or *Cochlospermum angolensis* used solely or mixed with different fruit, plant or algae extracts, was studied using different formulations (bags, leaves, roots, granulates, powders, liquids) and different preparation methods. Results of this work indicated that antioxidant activity and antioxidant compounds are significantly affected by the formulations and preparation methods. Babajide., *et al.* [30] studied a blend of equal portions of cucumber (50%) and pineapple (50%) juice that was mixed with clove and ginger powder spice with various concentrations to develop a new fruit drink that has health benefits. These blends are good

sources of phytochemicals such as alkaloid, flavonoid, saponin, tannin, terpenoid and phlobatannins - which may lead to the discovery and development of new functional drink that provide health benefits. Recent research findings give evidence that tea polyphenols can protect against different stages of carcinogenesis. The main catechin in green tea (EGCG) is being viewed as a cancer chemopreventive agent, antiobesity, and a cardiovascular protective compound [31]. The antioxidant activity and beneficial health effects of the main polyphenol of green tea (EGCG) was enhanced upon conjugation with docosahexaenoic acid and the tetraester so formed was able to arrest colon cancer effectively [32].

A very few studies have been conducted to evaluate the effects of refresher drinks which are mixtures of medicinal herbs. Chen, *et al.* [33] reported the anti-oxidative activities and free radical scavenging activities of 3 health drinks containing 29 Chinese medicinal herbs that were formulated in a food processing pilot plant. Results of this study showed that water extracts of herbs and herbal drinks showed free radical scavenging activity; thus, it is suggested that manufactured herbal drinks are safe and can be served as health-promoting drinks for the public [33]. A variety of health drinks were formulated with various herbs such as ashwagandha, tulsi, mulethi, awala, shatavari, godharu, arjun, giloy, safed musli, kalimirchi, haladi and jaiphal as active ingredients with aqueous extract of Stevia rebaudiana as a natural sweetener. These formulated drinks showed effective antioxidant, antidiabetic, antihypertensive and antimicrobial activities [34]. A whey-based beverage prepared from pineapple and a bottle gourd juice in combination with an edible extract of herbal medicinal plants such as *Mentha arvensis* not only have a nutritional value, but also possesses therapeutic, prophylactic, antibacterial and organoleptic properties [35].

In our unique formulation of the refresher drink, it was enriched with many polyphenols from turmeric, ginger, green tea, and black pepper. The antioxidant activity of the polyphenols from the selected spice extracts have the potential to quench the initiation of reactive oxygen species produced in body metabolism and delivers a refresher drink with an ensured biological activity.

### Limitations of the study

While the presented data seem promising, testing the antioxidant potency of more formulas and inclusion of a variety of plant-derived extracts would have made this more comprehensive. However, such can serve as a platform for further biomarker studies of drink formulations.

### Conclusion

A refresher drink that contains a spice extract mixture of turmeric, ginger, black pepper, and green tea was successfully formulated. Results showed that such a drink possesses a valuable antioxidant activity, and in a dose-dependent fashion. Thus, this formulation can be considered as an oxidative stress relieving drink that would have benefits to human health.

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