FORMULATION AND EVALUATION OF ANTIOXIDANT ACTIVITY OF HEALTH DRINK.

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This article is available online at www.ssjournals.com

ABSTRACT

An antioxidant health drink was prepared by using various extracts of the fruits. It was further evaluated on the basis of stability studies. Other parameters like pH, viscosity, density, colour, odour and taste were also taken into consideration. In vitro free radical scavenging activity by using beta-carotenes bleaching & lipid per oxidation method for the formulation was performed. The inhibition activity of the health drink on the peroxide ion of linoleic acid was measured by folic thiocyanate method in comparison to methanolic extract of green tea, ginkobiloba, vit.E, and BHA as positive controls. The health drink formulation of various fruits exhibited strong antioxidant activity. The health drink also showed promising stability studies.

KEY WORDS: Free radicals, Health drink, Lipid peroxidation, Antioxidant, Beta – carotene.

INTRODUCTION

Health is the level of functional and metabolic activity of an organism at both the micro (cellular) & macro (social) level. In the medical field, health is commonly defined as an organism's ability to efficiently respond to challenges (stresses) and effectively restore and sustain a state of balance known as homeostasis. Health drink rich in antioxidants help to maintain the health and prevent degenerative diseases. A very few type of health drinks are available in the marker, seasonal fruits cannot be obtained in all seasons. Hence there is a need of cost effective, side effect free ,portable health drink for body builders, athletes and all working class of people to boost their energy level to perform the best.

Recent developments in biomedicals point out the involvement of free radicals in many diseases (1). Free radicals attack the unsaturated fatty acids in the biomembranes resulting in membrane lipid peroxidation, a decrease in membrane fluidity, loss of enzymes and receptor activity and damage to membrane proteins leading to cell inactivation (2). Free radicals also attack DNA and cause mutation leading to cancer (3). For these reasons antioxidants are of interest for the treatment of many kinds of cellular degeneration

(4).Antioxidants are compounds that inhibit or delay the oxidation process by blocking the initiation or propagation of oxidizing chain reactions. There are two basic categories of antioxidant namely synthetic and natural ones. Restriction on the use of synthetic anti-oxidants is being imposed because of their carcinogenicity (5, 6). Thus the interest in natural antioxidants has been increased considerably. As resources of natural antioxidants much attention has been paid to plants (7, 8). Especially, the antioxidants present in edible plants have recently been considered as food additives (9, 10).

In the present study the antioxidant activity of health drink prepared from various fruits by beta carotein and lipid peroxidation method along with its stability studies is performed.

MATERIAL AND METHODS

All the fruits were purchased from the local market of Durg and they were identified by the botanist. The fruits were washed under running tap water, hand peeled, decored, deseeded and the pulp blended using an electric blender (Kenwood, England). Water was added in the ratio of 1:2 (w/v, pulp/water) to facilitate the blending process. The pulp was filtered using a muslin cloth. About 10% sugar solution was added.

Ferric chloride ($FeCl_3$), Tween 40, beta carotene and BHA were purchased from sigma chemical company. Ammonium thiocyanata and other chemicals were purchased from market.The aqueous extract of all fruits namely sweet orange (*Citrus aurantium*, rutaceac), grapes (*Vitis vinifera*, Vitaceae), Apple (*Malus domestic*, rosaceae), carrot (*Daucus carota*, apiace), banana (*Musa paradisiacal*, Musaceae) were extracted by electric blender to collect the fresh juice. The juice was lyophilized to get dried juice powder. The dried juice powder of all fruits was dissolved in 100 ml of distilled water and mixed with honey base and sodium benzoate to get desired health drink formulation.

Rapid evaluation of antioxidant activity by beta-carotene bleaching method

The rapid evaluation of antioxidant activity of hexane, chloroform and methanolic extracts were determined according to the beta-carotene bleaching method (11,12). In this procedure the plant extracts, Vit.E and BHA were applied on TLC plates and after developing with a suitable solvent system, plates were sprayed with a beta-carotene solution and exposed to daylight until discoloring of the background (6h.) The active compounds were seen as orange color on the plate. Methanolic extracts of Green tea, *Ginkgo biloba*, Vit.E and BHA were used as positive controls. Extracts which showed strong antioxidant activity were subjected to further tests.

Antioxidant activity evaluation by ferric thiocyanate method

The antioxidant activity of hexane, chloroform and methanolic extracts were determined using ferric thiocyanate method (FTC) (13). In this method, 500µg of each sample was dissolved in EtOH and added to a reaction mixture containing 2.88 ml of 2.5% linoleic acid and 9 ml of 40mM phosphate buffer in a vial. The vials were incubated at 40°C for 96 hours. During incubation (each 12 h),

0.1 ml of each vial was diluted with 9.7 ml of 75% EtOH, 0.1 ml ammonium thiocyanate and 0.1 ml $FeCl_3$. The absorbance of samples was measured at 500 nm and the percent of inhibition was determined. Methanolic extracts of Green tea and Ginkgo were used as positive controls with the same concentration. Ethanol with sample and without reagents was used as negative control. BHA and alpha tocopherol were used as positive controls.

Stability studies

The antioxidant health drink was kept in BOD incubator for 8 weeks in alternate light and dark cycles at various temperatures like 5^{o}C (refrigeration), and 28^{o}C (ambient). There was no change in consistency, appearance, taste, and found completely safe for consumption promising its stability studies.

Other Physiochemical and Organoleptic parameter which were evaluated for health drink are

1. pH-4.5
2. Viscocity – 1.57 cps
3. Density –1.2 g/ml
4. Colour – dark brown
5. Odour – sweet
6. Taste- characteristics

Statistical analysis

The collected data were subjected to appropriate statistical test like one-way ANOVA (Analysis of variance), followed by an appropriate turkey test. P values of less than 0.01 were considered as significant. The analysis was carried out using Graph pad prism software of version 4.

RESULTS AND DISCUSSION

Antioxidant activity by beta-carotene bleaching method:

The developed TLC plate after spraying with the reagent of beta-carotene showed discolorisationof the background after 6 hours, while the health drink showed orange band.

Antioxidant activity by ferric thiocyanate method:

Table 1- lists the antioxidant activity of the health drink with strong antioxidant activity. Health drink has shown to be more active antioxidant than *Ginkgo biloba* and alpha tocopherol. Table 1 shows the antioxidant activity of health drink in the linoleic acid peroxidation system (ferric thiocyanate method). The results indicate that health drink significantly ($p<0.05$) inhibits the linoleic acid peroxidation compared to the negative control.

Stability studies

There was no change in consistency, appearance, odour, taste and pH of health drink after its stability studies.Other Physiochemical and Organoleptic parameter which were evaluated for health drink were pH-4.5, Viscocity – 1.57cps, Density –1.2 g/ml, Colour – dark brown, Odour – sweet, Taste-characteristics

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Table 1-Antioxidant activity of Health drink measured by the ferric thiocyanate method after 60h incubation.

Sample	Absorbance at 500 nm	Percent of inhibition[a]
Control	1.050 ± 0.016	0.00
Health drink	0.125± 0.034	87.064*
Methanolic ex. of Green tea	0.053± 0.014	94.228*
Methanolic ex. of Ginkgo biloba	0.294 ±0.023	70.248*
alpha Tocopherol	0.217 ±0.012	77.139*
Butylated hydroxy anizole (BHA)	0.003 ±0.002	99.203*

[a]percent of inhibition (capacity to inhibit the peroxide formation in linoleic acid) =

[1- (absorbance of sample at 500 nm) / (absorbance of control at 500 nm] x 100.

A high inhibition percent indicates a high antioxidant activity.

Results are presented as mean + standard deviation(n=5).

* statistically significant ($p < 0.05$).