International Journal of Phytopharmacy
Vol. 5 (6), pp.139-143, Nov-Dec 2015
ISSN: 2277-2928 (Online)
Journal DOI: 10.7439/ijpp

www.ssjournals.com

Research Article

Oxidative stress and antimicrobial activity of formulated health drink

H. J. Dhongade[1*] and D. C. Goupale[2]

[1]*Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg, Chhattisgarh*
[2]*Bajiraoji Karanjekar College of Pharmacy, Sakoli, Dist: Bhandara. Maharashtra-441802*

*Correspondence Info:
H. J. Dhongade
Shri Rawatpura Sarkar Institute of Pharmacy,
Kumhari, Durg, Chhattisgarh
E-mail: hemantdhongade@gmail.com

Abstract

A health drink was prepared by using various extracts of the fruits like sweet orange, grapes, Apple, carrot, banana. It was further evaluated for antioxidant activity, antimicrobial activity and stability studies. Physiochemical and Organoleptic parameter were evaluated 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 antimicrobial activity was performed by Agar disk diffusion and Agar ditch diffusion methods using various strains of bacteria. Microbial growth was determined by measuring the diameter of the zone of inhibition. The health drink formulation of various fruits exhibited strong antioxidant activity and moderate antimicrobial activity. The health drink also showed promising stability studies.
Keywords: Health drink, Lipid peroxidation, Antioxidant, Antimicrobial, Beta–carotene.

1. 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 antioxidants 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].

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Systematic studies among various pharmacological compounds have revealed that any drug may have the possibility of possessing diverse functions and thus may have useful activity in completely different spheres of medicine.

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

2. Materials 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 laboratory. Ammonium thiocyanate 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.

2.1 Rapid evaluation of antioxidant activity by beta-carotene bleaching method:

The rapid evaluation of antioxidant activity of health drink and methanolic extracts of control drugs were determined according to the beta-carotene bleaching method [11, 12]. In this procedure the health drink, 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 colour 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.

2.2 Antioxidant activity evaluation by ferric thiocyanate method:

The antioxidant activity of health drink and methanolic extracts of control drugs 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. BHA and alpha tocopherol were used as positive controls.

2.3 Antimicrobial assay:

2.3.1 Test microorganisms

The microbial strains are identified strains and were obtained from the National Chemical Laboratory (NCL), Pune, India. The bacterial strains studied are Pseudomonas testosteroni NCIM 5098, Staphylococcus epidermidis ATCC 12228, Proteus morganii NCIM 2040, Bacillus subtilis ATCC 6633, Micrococcus flavus ATCC 10240 and Klebsiella pneumoniae NCIM 2719.

2.3.2 Assay

A loop full of the strain was inoculated in 30 ml of nutrient broth in a conical flask and incubated on a rotatory shaker for 24 h to activate the strain. Mueller Hinton Agar No. 2 was prepared for the study. The assay was performed using 2 methods. Agar disk diffusion [14] and Agar ditch diffusion [15] methods. The media and the test bacterial cultures were poured into Petri dishes (Hi-Media). The test strain (0.2 ml) was inoculated into the media (inoculum size 108 cells/ml) when the temperature reached 40-42˚C. Care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions. For the Agar disk diffusion method,

the test compound (0.2 ml) was introduced onto the disk (0.7 cm) (Hi-Media) and then allowed to dry. Thus the disk was completely saturated with the test compound. Then the disk was introduced onto the upper layer of the medium with the bacteria . The plates were incubated overnight at 37˚C. For the Agar ditch diffusion method, after the medium was solidified, a ditch was made in the plates with the help of a cup -borer (0.85 cm). The test compound was introduced into the well and the plates were incubated overnight at 37˚C.

2.4 Determination of the minimum inhibitory concentration (MIC):

Microbial growth was determined by measuring the diameter of the zone of inhibition. Standard antibiotic ciprofloxacin (Cadila Pharmaceuticals, India) at 2 µg/ ml concentration, was used as positive control. Distilled water was used as the control. The control activity was deducted from the test and the results obtained were tabulated.

2.5 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. Viscosity - 1.57 cps
3. Density - 1.2 g/ml
4. Colour - Dark brown
5. Odour - Sweet
6. Taste - Characteristics

2.6 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.

3. Results and discussion

3.1 Antioxidant activity by beta-carotene bleaching method

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

3.2 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 control drugs.

Table 1: Antioxidant activity of Health drink measured by the ferric thiocyanate method after 60 h 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 anisole (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 ($p < 0.05$).

3.3 Antimicrobial assay:

From Table 2, the health drink showed good antibacterial activity against Bacillus subtilis and kiebsiella pneumonia where it showed 75 % inhibition as compared to control. It showed satisfactory antibacterial activity against Proteus morganii and Micrococcus flavus where it showed 50 % inhibition. But health drink did not show any inhibition for Pseudomonas testosterone and Staphyllococcus epidermidis. The results of the study confirm the antimicrobial potential of the health drink. However, further detailed *invivo* studies are required.

Table 2: Effect of health drink on the growth of bacterial isolates:

Bacterial species (ATCC NO.)	Control	Ciprofloxacin	Test Extract
Pseudomonas testosterone (NCIM 5098)	++++	-	++++
Staphylococcus epidermidis (ATCC 12228)	++++	-	++++
Proteus morganii (NCIM 2040)	++++	-	++
Bacillus subtilis (ATCC 6633)	++++	-	+++
Micrococcus flavus (ATCC 10240)	++++	-	++
Klebsiella pneumonia (NCIM 2719)	++++	-	+++

Extent of growth: - Complete inhibition; + 75% inhibition; ++ 50% inhibition; +++ 25% inhibition; ++++ no inhibition.

3.4 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, Viscosity – 1.57cps, Density –1.2 g/ml, Colour – Dark brown, Odour – Sweet, Taste – Characteristics.

Acknowledgement

The researchers acknowledge Dr. P. Kashyap, Principal, and the management of Shri Rawatpura Sarkar Institute of Pharmacy for their keen interest and support provided during the course of this study.

References:

[1] Ames B N, Shigenaga M K and Hagen T M, Oxidants, antioxidants and the generative disease of aging*, Proc. Natl. Acad. Sci. U.S.A*., 1993; 90: 7915-7922.

[2] Dean R T and Davies M J, Reactive species and their accumulation on radical damaged proteins, *Trends Biochem. Sci*., 1993; 18: 437-441.

[3] Ceruti P, Oxy-radicals and cancer, *The Lancet,* 1994; 344: 862-863.

[4] Tutour B L, Antioxidative activities of algal extracts and Synergistic effect with vitamin E, *Phytochem*., 1990; 29: 3759-3765.

[5] Bronen A L, Toxicology and biochemistry of butylated hydroxy anizole and butylated hydroxy toluene, *J.Am. Oil. Chem. Soc*., 1975; 52: 59-63.

[6] Ito N, Fukushima S, Hasegawa A, Shibata M and Ogiso T, Carcinogenicity of butylated hydroxyl anisole in F344 rats, *J. Natl. Cancer Inst*., 1983; 70: 343-344.

[7] Couladis M, Tzakou O and Verykokidou E, Screening of some Greek aromatic plants for antioxidant activity, *J. Phytother. Res*., 2003; 17: 194-196.

[8] Linn C C, Wu S J and Chang C H, Antioxiant activity of *Cinammomum cassia*, *J. Phytother. Res*., 2003; 17: 726-730.

[9] Fukuda Y, Osawa T and Namiki M, Studies on antioxidant substance in sesame seeds, *Agric. Biol. Chem.* 1985; 49: 301-306.

[10] Igarashi K, Itoh M and Harada T, Major antioxidative substances in leaves Atsumi-Kabu (Red turnip, *Brassica comoestris* L.), *Agric. Biol. Chem*., 1990; 54: 1053-1055.

[11] Taga M S, Miller E F and Pratt D E, Chia seeds as a source of natural antioxidants, *J. Am. Oil. Chem. Soc*., 1984; 61: 928-931.

[12] Rajesh L and Mehta Joseph F, Ajowan as a source of natural lipid antioxidants, *Agric. Food Chem*., 1994; 42: 420-1422.

[13] Masude T, Isobe J, Jitoe A and Nakamati N, Antioxidative curcuminoids from rhizomes of *Curcuma xanthorrhiza, Phytochem*., 1992; 31: 3645- 3647.

[14] Salie F, Eagles P F K, Leng H M J, Preliminary antimicrobial screening of four South African Asteraceae species, *J Ethanopharmacol,* 1996; 52: 27-33.

[15] Perez C, Paul M, Bazerque P, Antibiotic assay by agar-well diffusion method, *Acta Biol Med Exp,* 1990; 15: 113-115.