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Antioxidant activity of a salt–spice–herbal mixture against free radical induction

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Abstract

A combination of spices (*Piper nigrum*, *Piper longum* and *Zingiber officinale*), herbs (*Cyperus rotundus* and *Plumbago zeylanica*) and salts make up *Amrita Bindu*. The study was focused to evaluate the antioxidant property of individual ingredients in *Amrita Bindu* against the free radical 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). The analysis revealed the antioxidant potential of the ingredients in the following order: *Piper nigrum* > *Piper longum* > *Cyperus rotundus* > *Plumbago zeylanca* > *Zingiber officinale*. Two different experiments were designed. In experiment I, rats were fed with normal diet whereas in experiment II rats were given feed mixed with *Amrita Bindu* for 3 weeks (4 g/kg of feed). Rats from both experimental groups were challenged against a single intraperitonial injection of phenylhydrazine (PHZ) (7.5 mg/kg body weight). At the end of 24 and 72 h, blood was analysed for free radicals and antioxidant levels. It was interesting to note that rats with *Amrita Bindu* pretreatment showed significantly lower levels of free radicals, lipid peroxidation and protein carbonyls along with significantly higher levels of antioxidants when compared with rats without *Amrita Bindu* pretreatment on PHZ administration. These results reveal that *Amrita Bindu*, a salt–spice–herbal mixture exerts a promising antioxidant potential against free radical induced oxidative damage.

Keywords: *Amrita Bindu*; Phenylhydrazine; Free radicals; Antioxidants

1. Introduction

In recent years, phytochemical constituents of plants with varied pharmacological, physiological and biochemical activity have received attention. Spices and herbs are part of the daily food in several parts of the world. In India, the dietary inclusions of peppers, ginger, coriander, cardamom, dill, turmeric, onions, etc. have been practiced for centuries. Diets rich in bioactive phytochemicals reduce the risk of degenerative disorders such as cancer, diabetes, cardiovascular and oxidative dysfunction (Youdim and Joseph, 2001; Bazzano et al., 2003; Sherry et al., 2003; Lee et al., 2004). Foods containing these phytochemicals not only can provide our diet with certain antioxidant vitamins like Vitamin C, Vitamin E and pro Vitamin A but also a complex mixture of other natural substances with antioxidant capacity.

Amrita Bindu is a salt–spice–herbal mixture formulated by Shanmugasundaram et al. (1994) based on Indian system of medicine. The ingredients present in *Amrita Bindu* are reported to have one or more pharmacological or biological effects useful for controlling the development of inflammatory and degenerative disorders and slowing the aging process, according to the Indian system of medicine (Ayurveda and Siddha). The five salts induppoo, kalluppoo, moongiluppoo, valayaluppoo, vengaram or borax present in *Amrita Bindu* provide chlorides, sulphates, phosphates, iron, carbonates of calcium, zinc, magnesium, sodium, potassium, borax, sulphides and trace elements. These are used in the treatment of enlargement of spleen and liver, dyspepsia, indigestion, urinary disorders and hemorrhoids. These are reported to be safe (Nadkarni, 1954a,b,c). The three spices *Piper nigrum*, *Piper longum* and *Zingiber officinale* present in *Amrita Bindu* contain active principles like piperine, piperidine and gingerberin which are known for hepato protective and antioxidant activity (Koul and Kapil, 1993; Khajuria et al., 1998; Surh, 1999). The herbs *Cyperus rotundus*, *Plumbago zeylanica* in *Amrita Bindu* are known for inhibitory

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effects against superoxide and nitric oxide production (Seo et al., 2001), chemopreventive agent against intestinal neoplasia (Sugie et al., 1998).

Amrita Bindu was found to combat free radical insults and to provide protection against lipid peroxidation and antioxidant depletion in nitrosamine-induced tissue damage studied in albino rats (Shanmugasundaram et al., 1994). *Amrita Bindu* has also been tested as a supplement in diabetic retinopathy and has been found to raise the antioxidant levels in blood and prevent further damage of the retina (Parthiban et al., 1996). *Amrita Bindu* has been used as an antioxidant therapy in asthmatic children (Kumar and Shanmugasundaram, 2004). Also the protective role of *Amrita Bindu* against aflatoxin B_1 induced oxidative damage to lipids and proteins in fish (labeo rohita) have been studied (Madhusudhanan et al., 2004). The present study was therefore designed to assess the antioxidant activity of individual ingredients in *Amrita Bindu* using 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) as a free radical inducer as well as to test its antioxidant potential in vivo using phenylhydrazine, free radical inducer in male albino rats. This will provide a rational basis for its use in phytomedicine as a positive health food supplement against any disease involving free radicals.

2. Materials and methods

2.1. Plant materials

Amrita Bindu is a gift from E.R.B. Shanmugasundaram Herbal Research Foundation, Chennai. *Amrita Bindu* is a salt–spice–herbal mixture and the ingredients present in it are (1) induppoo or saindhawa, (2) kalluppoo (rock salt), (3) moongiluppoo (pearl ash), (4) valayaluppoo (bangle salt), (5) karuuppoo (black salt), (6) vengaram (crude borax), (7) *Tribulus terrestris* L. (small caltrops), (8) *Calatropis gigantean* R.Br. (gigantic swallow wort and mudar), (9) *Zingiber qfficinale* (ginger), (10) *Piper longum* L. (dried catkins and long pepper), (11) *Piper nigrum* L. (black pepper), (12) *Plumbago zeylanica* L. (Ceylon leadwort), and (13) *Cyperus rotundus* L. (nutgrass). Its formula and its detailed preparation is described in detail by Shanmugasundaram et al. (1994). *Amrita Bindu* is reported to have no hepato or nephrotoxicity when administered for long periods.

2.2. Separation of lipophilic from hydrophilic antioxidants in Amrita Bindu/its ingredients

One gram of *Amrita Bindu*/or its constituents were weighed, to which was added 2 mL of 50 mM sodium phosphate buffer and 5 mL of ethyl acetate. Grinding was done using pestle and mortar to enable efficient extraction. This was transferred to a decantation funnel and the solid residue was discarded. The aqueous phase was collected to measure hydrophilic antioxidant activity (HAA) and the organic phase was collected to measure lipophilic antioxidant activity (LAA). All extraction procedures were carried out at 4 °C and fractions were analysed immediately.

2.3. Total antioxidant activity

The total antioxidant activity was measured using the method of Cano et al. (1998) with some modifications as in Arnao et al. (2001) using ABTS as a free radical inducer. Total antioxidant activity (TAA) is a parameter that quantifies the capacity of a sample (in either a natural or processed product) to scavenge free radicals in aqueous media. The 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical ($ABTS^{\bullet+}$) is generated enzymatically by horse radish peroxidase (HRP) in a typical peroxidative reaction in the presence of H_2O_2

$$ABTS + H_2O_2 \xrightarrow{HRP} ABTS^{\bullet+} + 2H_2O$$

The antioxidant activity assay was based on the preformed radical cation $ABTS^{\bullet+}$ reduction, measured by the decoloration (loss of absorbance) at 730 nm after the addition of the sample.

2.4. Assay of hydrophilic antioxidant activity

1.0 mL of reaction mixture contained 0.3 mL of ABTS, 0.35 mL of H_2O_2 and 0.2 mL of HRP in 0.15 mL of sodium phosphate buffer (pH 7.5) to give a final concentration of 2 mM, 15, 0.25 μM, 50 mM of ABTS, H_2O_2, HRP and sodium phosphate buffer, respectively. The reaction was monitored at 730 nm until stable absorbance was obtained. Then 50 μL of the aqueous phase was added to the reaction medium and the decrease in absorbance was determined after 5 min. Ascorbic acid was used as the standard. The hydrophilic antioxidant activity was expressed as mg/g *Amrita Bindu*/or its ingredients.

2.5. Assay of lipophilic antioxidant activity

1.0 mL of reaction mixture contained 0.35 mL of ABTS, 0.35 mL of H_2O_2 and 0.15 mL of HRP in 0.15 mL of pure ethanol to give a final concentration of 1 mM, 15, 6 μM of ABTS, H_2O_2, and HRP, respectively. The reaction was monitored at 730 nm until stable absorbance was obtained. Then 50 μL of the organic phase was added to the reaction medium and the decrease in absorbance was determined after 5 min. α-Tocopherol was used as the standard. The lipophilic antioxidant activity was expressed as mg/g *Amrita Bindu*/or its ingredients.

The total antioxidant activity of *Amrita Bindu*/or its ingredients was arrived at by adding the hydrophilic and lipophilic antioxidant activities of the same.

2.6. Animals

Male wistar strain rats were procured from the inbred stock of the Centre for Animal Health Studies, TANUVAS, Madhavaram, Chennai, India. They were housed in well-ventilated polyurethane cages with a 12 h light/12 h dark cycle, and received a standard rat chow (Amrut Laboratory Animal Feed, Bangalore). All procedures complied with the standards for the care and use of animal subjects as stated in the guidelines laid by Institutional Animal Ethical Committee (IAEC), University of Madras, Taramani Campus, Chennai.