INNOVARE JOURNAL OF LIFE SCIENCE

Vol 6, Issue 1, 2018

ISSN - 2321-550X

Research Article

ANTIOXIDANT AND ANTIHEMOLYTIC ACTIVITY OF *AVERRHOA BILIMBI* EXTRACT

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Received: 02 February 2018, Revised and Accepted: 19 February 2018

ABSTRACT

Objectives: The present investigation attempts to study the antioxidant and antihemolytic activity of ethyl acetate fraction of the bilimbi extract (BE) and determine the contributory phytochemicals.

Methods: Fresh fruits of *Averrhoa bilimbi* were dried and subjected to 60% aqueous methanol extraction followed by biphasic extraction with ethyl acetate and water. The ethyl acetate fraction BE underwent phytochemical screening, analyzed using reverse-phase high-performance liquid chromatography (RP-HPLC), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) radical scavenging activity, hydroxyl radical scavenging activity, red blood cell (RBC) protection activity, and DNA protection activity.

Results: In phytochemical screening analysis, we detected lipids, while alkaloids, flavonoids, saponins, terpenoids, cardiac glycosides, reducing sugars, and amino acids were not detected. HPLC analysis showed prominent peaks at 23, 27, and 37 min under 310 nm. The fraction expressed $ABTS^{\cdot+}$ radical scavenging activity, hydroxyl radical scavenging activity, RBC protection activity, and DNA protection activity, wherein TEAC value was 11.5 µM, and IC_{50} value of hydroxyl RSA and antihemolytic activity were 49 and 47 µg/ml, respectively.

Conclusion: The ethyl acetate fraction of bilimbi predominantly comprised lipids which exhibited significant antioxidant and protective properties.

Keywords: Antioxidant, DNA protection activity, Antihemolytic activity.

INTRODUCTION

The human body hosts a complex system of natural enzymatic and non-enzymatic antioxidant defenses which render protection from the harmful effects of free radicals and other oxidants. Free radicals include hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot-}$), nitric oxide ($NO^{\cdot}$), nitrogen dioxide ($NO_2^{\cdot}$), peroxyl ($ROO^{\cdot}$), and lipidperoxyl ($LOO^{\cdot}$) radicals while oxidants which are non free radicals but can easily lead to free radical reactions in living organisms include hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen (O_2), hypochlorous acid (HOCl), nitrous acid (HNO_2), peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), and lipid peroxide(LOOH) [1]. ROS and RNS are required at low or moderate concentration for the maturation process of cellular structures. They also function as weapons for the host defense system, wherein phagocytes (neutrophils, macrophages, and monocytes) release free radicals to destroy invading pathogenic microbes as a part of the body's defense mechanism against disease [2]. However, if produced in excess, free radicals tend to react with various organic substrates such as carbohydrates, lipids, proteins, and DNA [3]. The resultant has contributed to a large number of diseases including atherosclerosis, cancer, cardiovascular disease, neural disorders, Alzheimer's disease, Parkinson's disease, alcohol-induced liver disease, ulcerative colitis, and aging [4-6]. Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention and postponing the onset of degenerative disorders [7]. Following up the harmful tendencies of free radicals, indigenous fruits such as that of *Averrhoa bilimbi* are targets of potential sources of antioxidants and therapeutic components. *A. bilimbi* L. (Oxalidaceae), a native of Malaysia and Indonesia, is a widely cultivated tree in southern India, particularly in Mangalore and Udupi. Commonly known as bilimbi, the edible fresh oblong very sour fruits undergo production of vinegar, wine, pickles, jams, and jellies. Bilimbi is also medicinally used as a folk remedy for many symptoms and diseases such as fever, mumps, pimples, inflammation of the rectum and diabetes, itches, boils, rheumatism, syphilis, bilious colic, whooping cough, hypertension, stomach ache, and ulcer and as a cooling drink [8]. Experimental pharmacological studies have shown that the fruit alleviates hypertension while aqueous extract of fresh bilimbi shows low antioxidant activities and low nitric oxide inhibition activity. Interestingly, the ethyl acetate extract of the fruit has expressed significant antioxidant activity [9]. Our earlier findings using aqueous methanol extract of bilimbi have also shown significant antioxidant activity and expressed proapoptotic and antiangiogenic activity against Ehrlich ascites carcinoma cells *in vivo* [10,11]. The following investigation is thus the first report to study the antioxidant and antihemolytic activity of ethyl acetate fraction of the aqueous methanol bilimbi extract (BE) after its subjection to biphasic extraction and determining its contributory phytochemicals.

MATERIALS AND METHODS

2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), sodium dihydrogen phosphate, disodium hydrogen orthophosphate, Trolox (6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid), Tris, 2-deoxy-D-ribose, potassium dihydrogen orthophosphate, potassium hydroxide, ferric chloride, ascorbic acid, thiobarbituric acid, trichloroacetic acid, potassium ferricyanide, sodium chloride, agarose, and ethidium bromide were obtained from Himedia, India; hydrogen peroxide, glacial acetic acid, methanol, and lambda phage DNA were purchased from Merck, India.

Plant extraction preparation

A. bilimbi fruits were collected from Mangalore, Karnataka. Dr. Krishna Kumar H.N., Department of Botany, Pooja Bhagavat Memorial Mahajana Education Centre, Mysuru (Voucher No. 2017/11/01), authenticated the fruits deposited as voucher specimens at the Herbarium Repository, Department of Botany, PBMMEC, Mysuru. 1 kg of diced fruits dried at 50°C after thoroughly washing it. The dried fruits were ground to a fine powder. 10 g of the dried fruit powder underwent extraction under continuous agitation for 3 h at 40°C using 60% methanol in water followed by filtration using muslin cloth and drying of filtrate at 50°C. The aqueous reconstituted dried crude extract was centrifuged at