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Comparative *In vitro* Free Radical Scavenging Activity of some Indigenous Plants

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ABSTRACT: In present study, the comparative *in vitro* free radical scavenging activities of methanol extracts from three indigenous medicinal plants viz. Amla, Bitter gourd and Neem were evaluated by 1, 1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging method. Ascorbic acid was used as the reference. All of the plant extracts exhibited promising antiradical effects in a concentration dependent manner. Bitter gourd was found to be most active followed by Neem and Amla showing almost similar activities.
Key words: Antioxidant, free radical, Amla, Bitter gourd, Neem.

INTRODUCTION

There is extensive evidence to implicate free radicals in the development of degenerative diseases. Oxygen free radicals are formed in tissue cells by various endogenous and exogenous causes such as metabolism, chemicals, and ionizing radiation. Approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All these radicals are known as reactive oxygen species (ROS) exert oxidative stress to the cells. When the generation of ROS overtakes the antioxidant defense of the cells the free radicals start attacking cellular proteins, lipids and carbohydrates leading to the pathogenesis of many disorders including arthritis and connective tissue disorders, liver disorders, neurodegenerative disorders, cardiovascular disorders, diabetes, chronic inflammation, mutagenesis, carcinogenesis and in the process of ageing[1,2].

Antioxidants provide protection for living organisms from damage caused by uncontrolled production of reactive oxygen species (ROS) and the concomitant lipid peroxidation, protein damage and DNA strand breaking[3]. Current interest is focused on the potential role of antioxidants and antioxidant enzymes in the treatment and prevention of atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus and several others diseases[4].

Antioxidants are added to a variety of foods to prevent or deter free radical induced lipid peroxidation, which is responsible for the development of off-flavors and the undesirable chemical compounds in food[5]. These ROS cause destructive and irreversible damage to the components of a cell, such as lipids, proteins and DNA[6]. Although normal cells possess antioxidant defense systems against ROS in the cells induces diseases such as cancer and aging[7].

ROS are formed and degraded by all aerobic organisms. ROS can readily react with most biomolecules including proteins, lipids, lipoproteins and DNA. Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive oxygen species, which are capable of oxidizing biomolecules, resulting in tissue damage and cell death[8]. When the mechanism of antioxidant protection becomes unbalanced by exogenous and endogenous factors, it results in

inflammation, diabetes, genotoxicity, cancer and accelerating aging[9].

Antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage. The most commonly used antioxidants are BHA, BHT, propyl gallate and tert-butyl-hydroquinone[10]. However, they have been suspected of being responsible for liver damage and carcinogenesis in laboratory animals[11]. Therefore, the development and use of more effective antioxidants is desired.

Traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their therapeutic principles. Plants produce antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity. In present study the *in vitro* free radical scavenging effects of some indigenous medicinal plants like Amla, Neem and Bitter gourd (Karela) were investigated. These plants are very well known in traditional systems of Indian medicines and there are several reports on their biological and clinical potential including antioxidant activity especially for Amla [3,12,13]. Here the authors intend to report the comparative antiradical potential of these plants obtained form commercial sources.

MATERIALS AND METHODS

PLANT MATERIALS

The fresh unripe fruits of Amla (*Emblica officinalis* Gaerth, Euphorbiaceae) and Bitter gourd or Karela (*Momordica charantia* Linn., Cucurbitaceae) and young leaves of Neem (*Azadirachta indica*, Meliaceae) were collected during the month of November 2006 from local market of Asansol, Burdhaman district of West Benagl state, India. The species were identified at Central National Herbarium of Botanical Survey of India, Shibpur, Howrah, West Bengal, India. The plant parts were first washed thoroughly with running tap water, then cut into small pieces and shade dried at temperature 21-24 °C and ground into coarse powders with mechanical grinder and stored in an air-tight container.

PREPARATION OF EXTRACTS

Powdered plant materials (150 g of each) were macerated with 400 ml of methanol at 21-24 °C temperature for 2 days with frequent shaking. After 2 days, the extracts were filtered and to the marc part 300 ml of the solvent was added and allowed to stand for next 2 days at same temperature for second time maceration (re-maceration) and after two days, again filtered similarly. The combined filtrates (extracts) were evaporated *in vacuo* at 40 °C by using a rotary evaporator and the dry extracts obtained for each plant were stored in refrigerator for future use.

REAGENTS AND CHEMICALS

1,1-diphenyl-2-picryl-hydrazil (DPPH) and L-ascorbic acid were procured from Sigma Chemical Co., USA. All other chemicals, reagents and solvents were of analytical grade available commercially.

FREE RADICAL SCAVENGING ACTIVITY EVALUATED BY 1, 1-DIPHENYL-2-PICRYL-HYDRAZIL

The free radical scavenging activity of all of the extracts were evaluated by 1,1-diphenyl-2-picryl-hydrazil (DPPH) according to the previously reported method[14]. Briefly, an 0.1 mM solution of DPPH in methanol was prepared, and 1 ml of this solution was added to 3 ml of the solutions of all extracts in methanol at different concentrations (5, 10, 15, 20, 25 µg/ml). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. Then their absorbances were measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10 UV: Thermo Electron Corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated by using the following formula:

DPPH scavenging effect (% inhibition) = $[(A_0 - A_1) / A_0) \times 100]$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicate and the results were averaged.

RESULTS AND DISCUSSION

The stable DPPH radical model is a widely used, relatively quick and precise method for the evaluation of free radical scavenging activity. The effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable nitrogen centered free radical containing an odd electron in its structure that can accept an electron or hydrogen radical to become a stable diamagnetic molecule[15]. The absorption maximum of a stable DPPH radical in methanol is 517 nm. Because of its odd electron DPPH gives a strong absorption at 517 nm in the visible region (deep violet colour). The decrease in absorbance of DPPH radical is caused by antioxidants, as the reaction between antioxidant molecules and radical progresses, results in the scavenging of the free radicals by hydrogen donation. As the electron becomes paired off in presence of a free radical scavenger, the absorption diminishes, thus the resulting decrease in absorbance is stoichiometric with respect to the number of electrons taken up[14]. It is visually noticeable that as a change in color from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidant activity

of antioxidants[16]. It has been reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect or scavenge them, forms the pathological basis of several chronic disease conditions[1,2].

After present investigation, based on the data obtained from this study, all the extracts were found to be effective free radical inhibitor or scavenger, as well as a primary antioxidant that reacts with free radicals, which may limit free radical damage occurring in the human body. The results are depicted in Figure 1 which illustrates a significant increase in the inhibition of DPPH radicals due to the scavenging ability of the extracts and reference (ascorbic acid). Free radical scavenging activity also increased with increasing concentrations of the extracts in the range of 5-25 µg/ml.

Based on the results of this study, it is clear that all of the test plant extracts have powerful *in vitro* free radical scavenging properties against DPPH model in a concentration dependent manner. Bitter gourd was found to be most potent followed by Neem and Amla showing almost similar activities at the concentrations tested (Fig 1). Nevertheless, ascorbic acid (reference) was the most potent, remarkably active at lower concentrations (1-5 µg/ml). It is well known that Amla fruit is a rich source of ascorbic acid[12]. Amla extract, however was found to be less active than pure ascorbic acid (reference) and the bitter gourd extract. It becomes evident that the antiradical activities of all the extracts are due to the presence of putative phytoconstituents such as triterpenoids and flavonoids present in Neem and Bitter gourd and ascorbic acid and tannins in Amla. The possible complex interaction of different chemical constituents in extracts may influence their free radical scavenging effects.

From the results of present study it can be concluded that Amla, Bitter gourd and Neem, all demonstrated promising *in vitro* free radical scavenging properties; Bitter gourd being the most active among them.

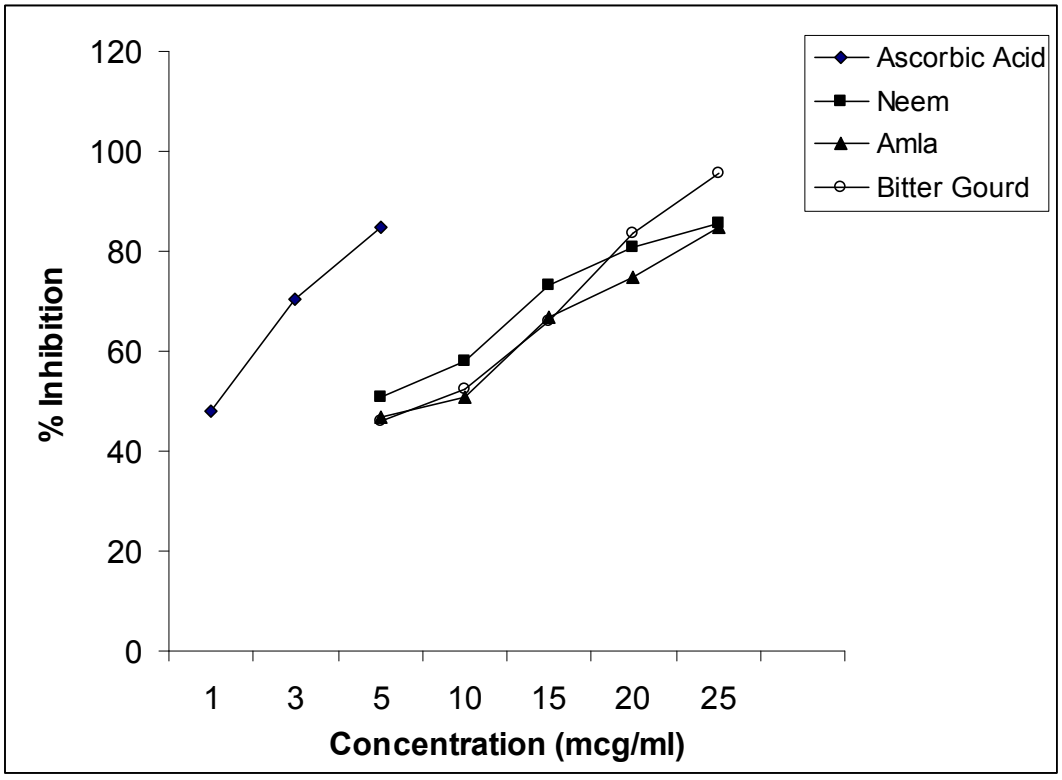

Fig. 1. Comparative free radical scavenging activity of Amla, Bitter gourd, Neem and Ascorbic acid (reference)

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