International Journal of Phytopharmacy
Vol. 1 (2), pp.50-54, Nov-Dec 2011

Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF *IPOEMA BILOBA*

Priti Tagde, Farukh Khan, Bhushan Gandhare

NRI Institute of Pharmacy Bhopal, M.P, India
Corresponding Author: tagde_priti@rediffmail.com

ABSTRACT

Biomolecules can be oxidized by free radicals. This oxidative damage has an important etiological role in aging and the development of diseases like cancer, atherosclerosis, and other inflammatory disorders. Synthetic antioxidants, like butylated hydroxyanisole, are good free radical scavengers; however, the synthetic antioxidants can be carcinogenic. Therefore, there is an increasing interest in searching for antioxidants of natural origin. Antioxidants with different chemical properties may recharge each other in an antioxidant network. The total antioxidant content of dietary plants may therefore be a useful tool for testing the 'antioxidant network' hypothesis. Several berries, fruits, nuts, seeds, vegetables, drinks and spices have been found to be high in total antioxidants. Initial studies in animals and humans are supportive as to the beneficial effects of dietary plants rich in total antioxidants. Additionally, antioxidants and other plant compounds may also improve the endogenous antioxidant defense through induction of antioxidant and phase 2 enzymes.[1,2] Dietary plants rich in such compounds include broccoli, brussel sprouts, cabbage, kale, cauliflower, carrots, onions, tomatoes, spinach and garlic , antioxidants and other plant compounds may also improve the endogenous antioxidant defense through induction of antioxidant and phase 2 enzymes.

Keywords- *Ipoema biloba*; Dimethyl Sulfoxide; Ethyl Diamine Tetra Acetic Acid; Thio Barbituric Acid

1. INTRODUCTION

Antioxidants are large category of nutritional & chemical substances that neutralizes free radical and convert it to a harmless molecule.[3,4,5,6] Free Radicals - Reactive species generated in & outside the body as a result oxidative degeneration. Many of our body's normal metabolic processes produce free radicals. Free radicals are also formed by enzymatic production. However, external sources such as pollution, cigarette smoke and sunlight cause the production of free radicals. [7,8]

Vitamins & Minerals as Antioxidants: Vitamins & minerals are growth & metabolism regulator. Vitamin A inhibit enzymes that promote cancerous growth, carotene prevent cervical, oral lung & prostate cancer. Vitamin C destroys free radicals & increase collagen synthesis, & produces cytotoxicity in cancerous cells. Minerals such as Selenium acts as an antioxidant by activating enzymes Glutathione peroxidase.[9,10,11]

High molecular weight compounds such as albumin, ceruloplasmin, transferrin, Hepatoglubulin & low molecular weight compounds as tocopherol, carotenoid, quinine, bilirubin etc. also acts as antioxidants.

Cancer Prevention – Integral Role of Nutrition: The role of diet in cancer prevention is now widely known. In one study, Willett and colleagues at Harvard University tracked the cancer rates of 80,000 American nurses while monitoring their diets. The study determined that colon cancer was closely associated with consumption of high amounts of animal fat, processed meats, and liver. Women who ate red meat every day had two-and-one-half times the colon cancer risk of those who ate it less than once a month. Not just the amount of fat, but also the type of fat in the diet, affects cancer rates. Results of a large-population study relating diet and heart disease suggest that those who consumed the most omega-3 fatty acids (found in fish oils and canola oil) had lower cancer rates than those who consumed more omega-6 fatty acids (found in corn, safflower, and sunflower oils). Moreover, vegetables and fruits have a demonstrable protective against many cancers – most likely due to high concentrations of vitamins, carotenoids, indoles, and other anticarcinogens.[12,13,14,15]

2. MATERIALS AND METHODS[8-14]:

2.1 Plant Material: The leaves of *Ipomoea biloba* were identified & Ipomoea was collected from Nursery in Misrod, Bhopal.

2.2 Extraction Protocols

Preparation of Extracts: Cold Maceration Process: About (100g) of coarsely powdered, air dried leaf powder were placed separately in a dried glass stoppered conical flask, the powder were soaked separately in the sufficient quantity of solvent (50% methanol) in the ratio of (1:10) drug:solvent ratio separately for 7 days in cool & dark place with occasional stirring. The mixtures were strained. The strained liquids were filtered separately. The marc was pressed with the tincture press for individual extract. The liquids were mixed & concentrated liquids were transferred to a tarred flat bottom dish & evaporated to dryness in hot air oven at 45-50^{o} C. The dried extracts were cooled in dessicator & weighed individually. The % yield was calculated for each extract on the basis of fresh & dried weight and found to be 8 %. [16,17]

2.3. In Vitro antioxidant activity

Various in vitro models

1. ***DPPH Method:*** (1,1 diphenyl 2 picryl hydrazyl)- reduction of methanolic solution of DPPH by free radical scavengers.[12]
2. ***Superoxide radical scavenging activity*** – measured by riboflavin, light, NBT (nitroblue tetrazolium) reduction.[18]
3. ***Hydroxyl radical scavenging activity*** – is by Fenton reaction which is to used in the present study of Antioxidant.[18]

Principle- Fenton reaction is used to generate OH^{-} radical in a test system & free radical scavenging activity was determined by degradation of deoxyribose. Fe3+-ascorbate- EDTA-H2O2 system produces OH- radical which reacts with deoxyribose & a set of reaction that results in the generation of Thio Barbituric Acid Reactive Substances (TBARS). Measurement of TBARS thus gives an index of free radical scavenging activity. Radical scavenging by Antioxidants will thus results in the inhibition of TBARS.[19]

Procedure

I. Hydroxyl radical scavenging activity

1. Reaction Mixture consisted of 100µl deoxyribose(3mM), 50µl $FeCl_3$(0.2mM), 50µl EDTA(0.1mM), 100µl H_2O_2 (mM)in 550µl PO_4 buffer saline(pH=7.4)
2. Plant extract at concentration 1,10,50,100, 200µl for spinach, ipomoea & coriander & 0.5,1,2.5,5,7.5,10 l for sandal was added to the reaction to make a final volume of 1ml.
3. Reaction mixture was incubated for 1 hr at room temperature.

4. Mixture was then incubated for 20 min in a boiling water bath with 0.5 ml 0f 3% TBA & TCA each, cooled & centrifuged.
5. The absorbance in the supernatant was measured at 532 nm in UV spectrophotometer.

The test tube with PBS was considered as blank & DMSO was used as positive control. The results are expressed as % inhibition of TBARS.

II. Prooxidant Activity: Several compounds that are antioxidants at higher concentration are known to act as prooxidant at lower concentration. This can be tested by measuring the degradation of deoxyribose in reacting mixture deprived of ascorbic acid .[19]

III. Ability to chelate iron: In the absence of EDTA, iron ions bind to deoxyribose molecule and being about site specific damage in the molecule in the presence of ascorbic acid and H_2O_2.[19]

3. RESULTS

3.1 Hydroxyl Radical Scavenging Activity: As shown in Fig(1.1) it showed % TBARS inhibition at all concentration 1,10,50,100 & 200µg but from but to lesser extent than DMSO at equimolar concentration & TBARS decreases linearly with increase in drug concentration. It indicates statistically extremely significant ($p<0.0001$) inhibitory effects at all concentration 1,10,50,100 & 200µg.

3.2 Prooxidant Activity*:* As seen from Fig (1.2) it showed marked inhibitory effects on TBARS in the absence of Ascorbic acid & decreases linearly with increase in concentration. At higher concentration 200µg % TBARS inhibition sharply decreases showing its prooxidant at higher concentration.

3.3 Chelate Iron: As shown in Fig(1.3) there was concentration dependent increase in % TBARS inhibitory effects in the absence of EDTA up to 50µg but decreases linearly at concentration 100 & 200µg but. Statistically it showed extremely significant inhibitory effects at all concentration ($p<0.0001$)

4. DISUSSION

Ipomoea biloba TBARS formation at concentration 1, 10, 50, 100, 200 µg as good as DMSO at equimolar concentration but to lesser extent; Hence they show Free Radical Scavenging Activity & can be concluded as Antioxidants.

Ipomoea biloba at concentration 200 µg inhibited TBARS formation when degradation of deoxyribose is measured devoid of Ascorbic acid. Hence they can be concluded to have prooxidant activity at higher doses.

Ipomoea biloba inhibited TBARS formation at concentration 1, 10, 50, 100, 200 µg in the absence of EDTA. . Hence they can be concluded to have Iron Chelating activity.

CONCLUSION

Ipomoea biloba, contains various crucial & potential phytochemicals, vitamins & minerals, enzymes etc. They not only exhibit antioxidant activity but also antiproliferative, anti-inflammatory, antiaging etc. in biological system. It contains several active compounds such as bioflavonoids which are known to have cancer prevention properties.

It inhibits growth of cancerous cells in breast, colon, prostate & lungs. These phytochemicals readily scavenge free radical which oxidizes biomolecules & cause oxidative damage. The oxidative damage has an important etiological role in aging & development of diseases like cancer, atherosclerosis, dementia & other inflammatory disorders.

Therefore *Ipomoea biloba* with their enormous potential to scavenge free radicals are excellently significant to have antioxidant activity.

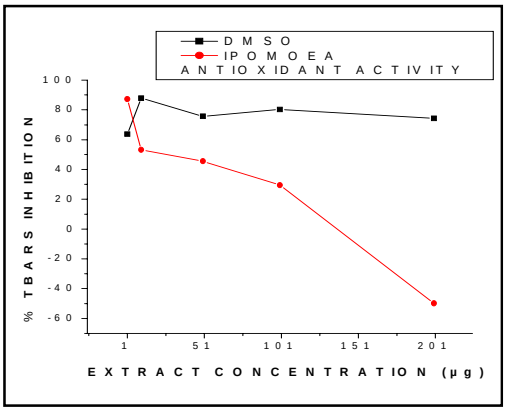

Fig (1.1) Hydroxyl Radical Scavenging Activity

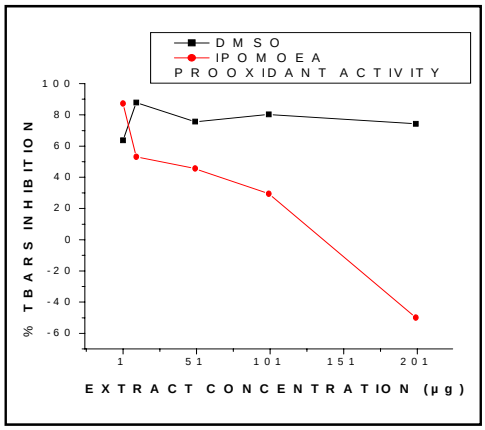

Fig (1.2) Prooxidant Activity

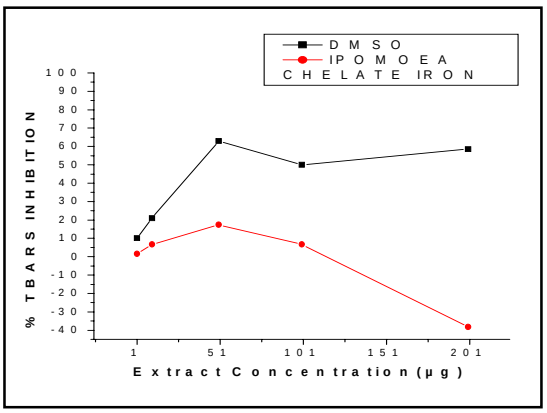

Fig (1.3) Chelate Iron

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