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Research Paper

# **IN VITRO INHIBITION OF MAO-A AND PLA ENZYMES AND ANTIOXIDANT ACTIVITY OF IMPORTANT MEDICINAL PLANTS EXTRACTS**

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To evaluate antioxidant and *in vitro* inhibition of MAO-A and PLA enzymes of medicinal plant extracts, the anti-oxidant assay was done by the following four different radical scavenging, viz., DPPH, Super oxide, Hydroxyl radical and reducing power. Amla extract showed the highest radical scavenging activity  $IC_{50} = 6 \mu\text{g/mL}$  value and the other two Gardenia  $IC_{50} = 9 \mu\text{g/mL}$  and Blueberry  $IC_{50} = 13 \mu\text{g/mL}$  exhibited similar activity. The PLA enzyme inhibitory assay was carried out and the Blueberry and Gardenia extract showed the highest  $IC_{50} = 46 \mu\text{g/mL}$  and  $38 \mu\text{g/mL}$ , respectively but less activity was observed in Amla extract. The MAO-A (monoamino oxidase) enzyme inhibitory assay was done and Blueberry and Gardenia extract inhibited this enzyme greatly when compared to Amla  $IC_{50} = 140 \mu\text{g/mL}$ ,  $116 \mu\text{g/mL}$  and  $90 \mu\text{g/mL}$ , respectively. The present investigation evidences that all the three plant extracts exhibited significant antioxidant activity. Amla and Gardenia were found to be good inhibitors of PLA and MAO-A enzymes which are involved in anti-inflammatory and depression, respectively.

**Keywords:** Anti-oxidant, Medicinal plant, MAO-A, PLA

## **INTRODUCTION**

Nutraceuticals, a term combining the words “nutrition” and “pharmaceuticals”, is a food or food product that provides health and medical benefits, including the prevention and treatment of disease. Plant-derived antioxidants have been shown to function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors, and synergists. Free radicals may be defined as chemical

species associated with an odd or unpaired electron. They are neutral, short lived, unstable and highly reactive to pair up the odd electron and finally achieve stable configuration. They are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, liver

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diseases, diabetes mellitus, inflammation, renal failure, brain dysfunction and stress among others (Ames *et al.*, 1993; Joseph *et al.*, 1999). Antioxidants are capable of stabilizing or deactivating, free radicals before reactive oxygen species attack cells and it is believed that higher intake of antioxidant rich food is associated with decrease risk of degenerative disease particularly Cardiovascular disease [Larson, 1998; Khopde *et al.*, 2001]. The plants evidence natural antioxidants in every part. These antioxidants include carotenoids, vitamins, phenols, flavonoids, dietary glutathione, and endogenous metabolites (Frei *et al.*, 1989). Several studies have reported on plant derived anti-oxidant Nutraceuticals Scavenging free radicals and modulate oxidative stress related degenerative effects. Nutraceuticals are becoming widely incorporated in function food owing to their therapeutic effects in enhancing the well being. There is a great deal of interest in newer natural bioactive molecules with health promoting potential. The three medicinally important plants Amla, Gardenia and Blueberry, contain certain chemical components (metabolites/ phytochemicals) that exert a potent pharmacological action in humans and animals.

**Amla:** Amla, botanically called as *Phyllanthus emblica* L. provides active levels of tannins contain gallic acid, ellagic acid also glucose and rich source of vitamin C (ascorbic acid) and polyphenols content (Shah and Bhattacharya, 1982). Ascorbic acid shows anti-oxidant and anti-inflammatory properties (El-Mekkawyt *et al.*, 1995; Jacob *et al.*, 1988). Amla also has antiviral, hyper cholesteromic, hypolipidemic, anticarcinogenic, hepatoprotective, immunomodulatory, and hypoglycemic activities (Thakur, 1985; Sultana *et al.*, 2008). It provides

protection for human dermal fibroblasts against oxidative stress. It is used for many chronic conditions including diabetes, for mental and memory effects. It is also used for dyspepsia, gastritis, hyperacidity, constipation, ulcerative colitis, anemia, diabetes, asthma, osteoporosis, neurasthenia, etc. The extracts of amla also have anti-microbial properties.

**Gardenia:** *Gardenia jasminoides* J. Ellis (Rubiaceae) is a white fragrant flower ever green tropical plant a favorite in gardens world wide. *G. jasminoides* has been included in traditional medicinal formulations for the treatment of inflammation, jaundice, head ache, edema, fever, hepatitis disorders and hypertension (Tseng *et al.*, 1995; Jagadeeswaran *et al.*, 2000; Koo *et al.*, 2004). It has effective pharmacological actions, as well as cytotoxic, anti-inflammatory actions and fibrolytic effects (Koo *et al.*, 2006; Oshima *et al.*, 1988; Wei *et al.*, 2008). The compounds geniposide, gardenoside, crocin, crocetin and gardenin are found (Yamaguchi *et al.*, 1998). The major components of gardenia fruit are geniposide and water soluble pigment crocins.

**Blueberry:** *Vaccinium corymbosum* L. fruit benefits for potential health due their bioactive compounds (polyphenols, including anthocyanins and other flavonoids and ascorbic acid) (Nishikimi *et al.*, 1972). Blueberries contain beta carotene, vitamin C and vitamin E. In addition carotenoids, anthocyanins, ellagic acid, folic acid and phenolics that can also act as anti-oxidants are found. The significance of their presence and modes of action remain largely unexplored.

In this investigation, anti-oxidant potential employing various *in vitro* assay system such as DPPH, super oxide, hydroxyl radical scavenging,

reducing power and also inhibition of some enzymes MAO-A, which are involved in depression and phospho lipase-A (inflammation), inflammatory (enzyme assay), anti-depressant (MAO) and polyphenols of the aqueous extract of Amla, Gardenia and Blueberry were studied.

## MATERIALS AND METHODS

**Chemicals required:** Nitro blue tetrazolium (NBT), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Phenazine Methosulphate (PMS), Thiobarbituric acid (TBA), EDTA, FeCl<sub>3</sub>, Deoxy ribose, H<sub>2</sub>O<sub>2</sub>, Ascorbic acid in Potassium phosphate buffer, Tri chloro acetic acid (TCA), Nicotinamide adenine di nucleotide reduced (NADH), Potassium ferricyanide {K<sub>3</sub>Fe(CN)<sub>6</sub>}, Sodium carbonate {Na<sub>2</sub>CO<sub>3</sub>}. PLA enzyme partially purified from plural fluid, MAO-A partially purified from rat brain, ursolic acid, fluoxetine. All reagents were analytical grade and UV-visible 1800-Shimadzu.

### Preparation of Plant Extracts

The plants selected were procured from the approved supplier. All the plants were washed with water, dried and then powdered. Extraction was carried out in large scale reactor, using water for Amla and Gardenia and 70% alcohol for Blueberry. Then the concentrated extracts were spray dried and the dried powder was used for further analysis, viz., antioxidant, and MAO-A and PLA inhibitor.

### Antioxidant Activity

#### DPPH (1, 1-Diphenyl -2- Picrylhydrazyl) Radical Scavenging Assays

DPPH (1, 1-Diphenyl -2- Picrylhydrazyl) Radical Scavenging Assays were performed in 3 ml reaction mixtures containing 1 mL of DPPH solution (0.1 mM/L, in 95% ethanol v/v) 50 µL of

different concentrations of the extract and 1.95 mL deionized H<sub>2</sub>O. The reaction mixture was shaken and incubated for 20 min at room temperature and absorbance was read at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and calculated using the following equation (Yen and Chen, 1995).

$$\text{Scavenging effect (\%)} = \{1 - A_{\text{sample}}(517 \text{ nm}) / A_{\text{control}}(517 \text{ nm})\} \times 100.$$

IC<sub>50</sub> represents the level where 50% of the radicals were scavenged by test samples.

### Super Oxide Radical Scavenging Assay

For assay of Super oxide Radical Scavenging, the reaction mixture containing different concentrations of extract, PMS (Phenazine Metho Sulphate) (0.1 mM/L), NADH (Nicotinamide Adenine Di nucleotide) (1 mM/L) and NBT (Nitro Blue tetra zolium) (1 mM/L) in phosphate buffer (0.1 mol/L, pH 7.4) was incubated at room temperature for 5 min. Read the absorbance at 560 nm against a blank. The Scavenging effect was calculated using the above equation.

### Hydroxyl Radical Scavenging Assay

For assay of Hydroxyl Radical Scavenging, the reaction containing different concentration of extract, Deoxy ribose (10 mM/L), H<sub>2</sub>O<sub>2</sub> (10 mM/L), FeCl<sub>3</sub> (5 mM/L) EDTA (1 mM/L) and Ascorbic acid (5 mM/L) in potassium phosphate buffer (50 mM/L, pH 7.4). The reaction mixture was incubated for 60 min at 37°C. The reaction was terminated by adding 5% of TCA (tri chloro acetic acid) followed by the addition of 0.2% TBA (Thio barbituric acid), boiled on water bath for 15 min. Absorbance of the color was measured at 535 nm against a blank, and inhibition of the oxidation of deoxy ribose was calculated with respect to

the control.

### Reducing Power Assay

Plant extract was dissolved in Phosphate buffer (0.2 moles, pH=6.6) with different concentrations to which 0.1 mL of Potassium ferric cyanide  $\{K_3Fe(CN)_6\}$  was added and made up to 1 mL with phosphate buffer and boiled at 50°C for 20 min. Then 3 mL of 10% TCA was added, centrifuged at 3000 rpm for 10 min. The supernatant was mixed with 1 mL of 0.1% Ferric chloride and absorbance was read at 700 nm.

### In Vitro PLA Assay

PLA assay was carried as Anti-inflammatory enzyme. The reaction mixture containing 0.2 mL of ether, 50 mM of Tris HCl (pH-7.4), 50 mM of calcium and enzyme was pre incubated for 15 min before adding substrate to the positive and inhibitor and vortex for 1 min. Then incubate at 37°C for 60 min. Add 0.5 mL of modified Dole's mixture to negative and then add substrate to it. Add 0.5 mL of modified Dole's mixture and 0.5 mL of petroleum ether, vortex for 1 min, centrifuge at 1000 rpm for 5 min at room temperature. Remove 0.5 mL of upper organic phase and transferred into a test tube containing 0.5 mL chloroform: petroleum ether (5:1) and freshly prepared cobalt reagent (1 mL). Then centrifuge at 1000 rpm for 15 mins at room temperature. Remove 0.5 ml of upper organic phase and transfer into a test tube (1-nitroso -2- naphthol, 0.4% in 96% ethanol). After 30 min, content is diluted to 3.35 mL by addition of 2 mL Methanol and read the absorbance at 530 nm against blank ursolic acid as a standard.

### In Vitro MAO-A Assay

The mixture contained 4 mM of 5 BHT and add 250  $\mu$ L of Mitochondrial fraction, 710  $\mu$ L of Sodium

Phosphate buffer (pH=7.4). Vortex for one min and incubate at 37°C for 60 min. Then add 1 mL 1 M HCl to stop the reaction. The reaction product is extracted with 5 mL of n-butyl acetate. The organic phase was measured at 242 nm. Fluoxetine is used as a standard.

### Determination of Total Polyphenols

Total Polyphenols assay was done. For which 30-40 mg of sample was dissolved in 50% methanol in 50 mL STD volumetric flask. Then take 5 mL sample, i.e., from the above and make up to 10 mL with 50% methanol in STD volumetric flask. Add 15 mL of 50% methanol in a vial, viz, Blank, 1, 2...etc. Add 1 mL of 50% methanol to blank and 1 mL of sample to the vials 1, 2....etc. Add 1 mL of phenol reagent to all the vials and 3 mL of 20% Sodium carbonate  $\{Na_2CO_3\}$  (Anhydrous). Keep it in hot boiling water bath at 45°C for 30 min. Then centrifuge for 5 min at 2000 rpm and absorbance was read at 545 nm.

$$\text{Percentage} = \frac{\text{Sample absorbance (a) - b/m} \times 50}{1000 \times \text{weight in grams}}$$

where m = Intercept (3.8421)

b = Slope (0.0549)

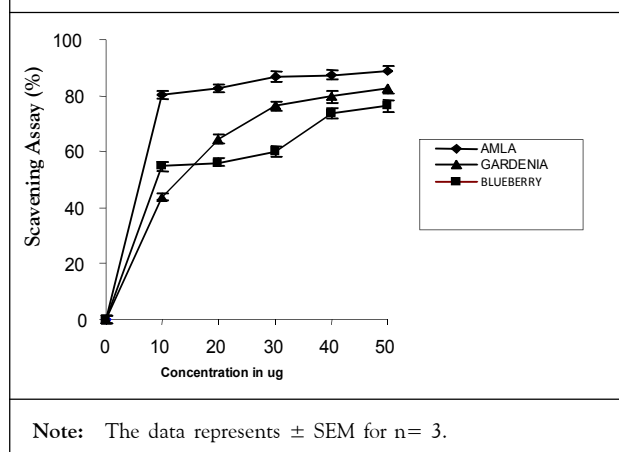
## RESULTS AND DISCUSSION

Extracts were subjected for the evaluation of activity and inhibition by using various *In vitro* assay systems and the  $IC_{50}$  values were calculated and depicted in the Table 1. Scavenging activity for free radicals of 1,1-diphenyl-2-picrylhydrazyl (DPPH) has been widely used to evaluate the antioxidant activity of natural products from plant and microbial sources. A high radical scavenging activity was observed in Amla than that of Gardenia and Blueberry extracts (Figure 1) in a concentration dependent manner. Proton

**Table 1: Antioxidant activity of Amla, Gardenia and Blueberry**

IC <sub>50</sub> in µg/ml					
Antioxidants activity					
Extracts	DPPH radical scavenging	Super-oxide radical scavenging	Hydroxyl radical scavenging	MAO-A	Enzyme assay PLA
Amla	6.0	5.5	6.0	90	-
Gardenia	9.0	6.0	7.0	116	38
Blueberry	13.0	7.0	7.5	140	46

**Figure 1: DPPH Radical Scavenging Activities of Amla, Gardenia and Blueberry extracts**

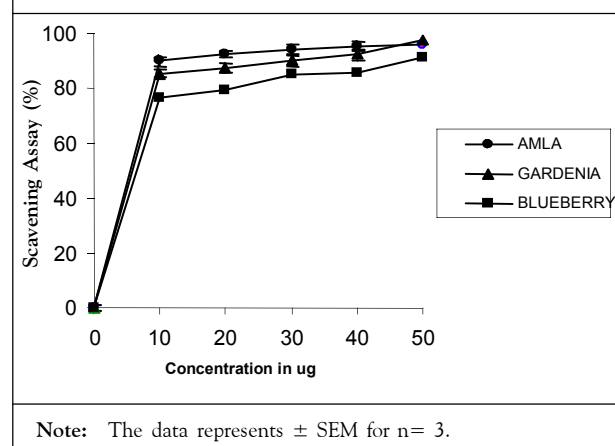


Although super oxide anion is by itself a weak oxidant, it gives rise to the powerful and dangerous hydroxyl radicals as well as singlet oxygen both of which contribute to the oxidative stress. Therefore super oxide radical scavenging by antioxidants has physiological implication. Amla and Gardenia showed similar activity and Blueberry slightly less than the other two (IC<sub>50</sub>= Amla-5.5 µg, Gardenia-6 µg and Blueberry-7 µg) (Figure 2). Anti-oxidant activity of Amla may be due to the presence of high levels of super oxide dismutase.

radical scavenging action is an important attribute of anti-oxidants, which is measured by DPPH radical scavenging assay. DPPH, a protonated radical, has characteristic absorbance maxima at 517 nm which decrease with the scavenging of the proton radical. Hydrogen donating ability of the anti-oxidant molecules contributes to its free radical scavenging nature (IC<sub>50</sub> for Amla-6 µg, Gardenia-9 µg and Blueberry-13 µg).

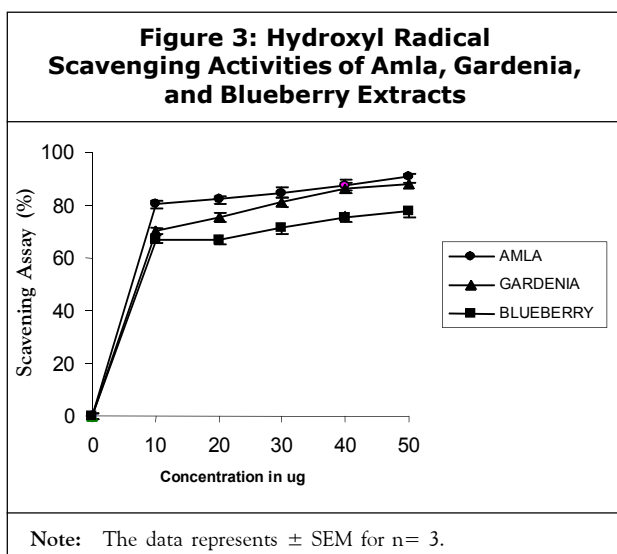
Super oxide anion is one of the most representative free radical and is generated during the normal physiological process mainly in mitochondria. In cellular oxidation reactions, superoxide radicals have their initial effects magnified because they produce other kinds of cell-damaging free radicals and oxidizing agents.

**Figure 2: Superoxide Radical Scavenging Activities of Amla, Gardenia and Blueberry Crude Extracts**



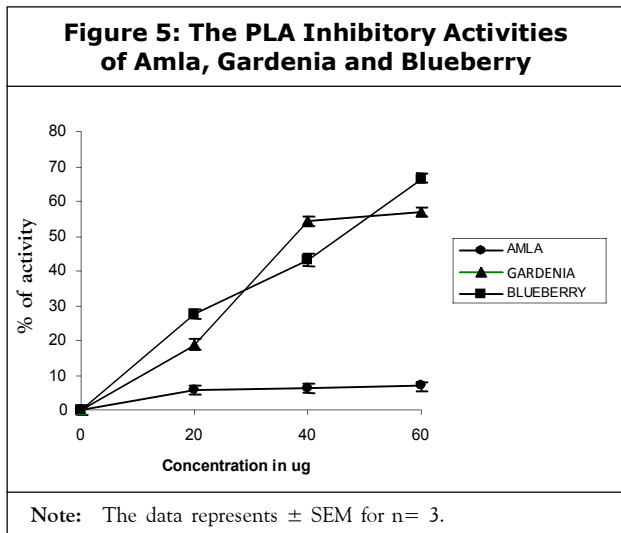
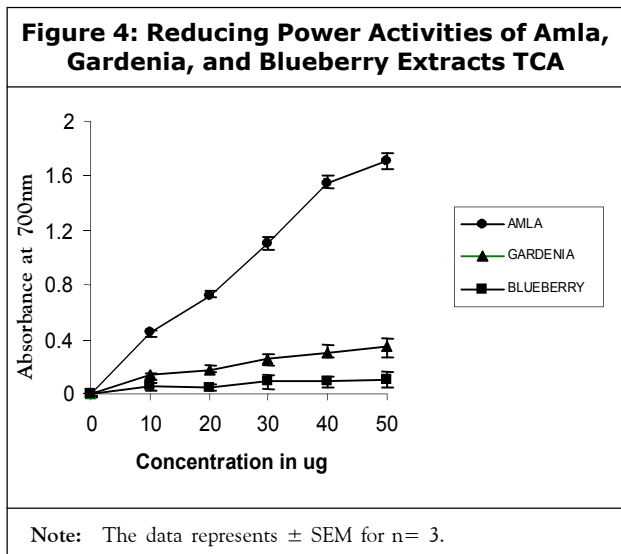
The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in

free radical pathology, capable of damaging biomolecules of the living cells. Hydroxyl radical has the capacity to cause DNA strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. Amla and Gardenia showed the highest and less activity was observed in Blueberry. ( $IC_{50}$  = Amla- 6  $\mu$ g, Gardenia- 7  $\mu$ g and Blueberry-7.5  $\mu$ g) (Figure 3).

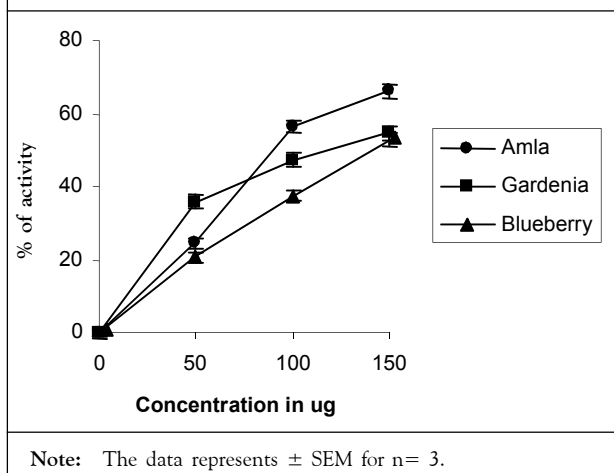


The reducing power of Amla, Gardenia and Blueberry extracts was concentration dependent. It is believed the anti-oxidant activity and reducing power are interrelated. There was increase in concentration of reducing power assay as shown in Figure 4.

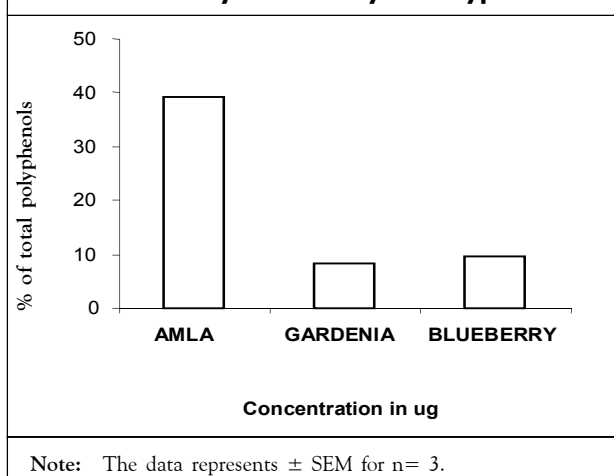
Phospho lipids are present in plasma membrane, which can be broken down by the enzyme PLA and releases free fatty acids. This free fatty acids enters the inflammatory pathway say for example if that free fatty acid is Arachidonic acid; it is acted by the cyclo-oxygenase 1 and 2 and leads to the generation of inflammatory (pain inducing) molecules like, prostaglandin and thrombin etc inhibition. PLA enzyme possesses anti-inflammatory activity. In this study Blueberry and Gardenia have shown more inhibition when compared to Amla (Figure 5).



Mono amine oxidases are enzymes that catalyze the oxidation of mono amines, particularly important in the catabolism of mono amines ingested in food. They are found bound to the outer membrane of mitochondria in most cell types in the body. It plays a role in the inactivation of neuro transmitters, MAO dysfunction is thought to be responsible for a number of neurological disorders. Mono amine oxidase inhibitors are one of the major classes of drug prescribed for the treatment of depression. In this study, Gardenia and Blueberry have higher inhibitory activity compared to Amla (Figure 6).

**Figure 6: Effect of Amla, Gardenia and Blueberry Extract on MAO-A activity**

Polyphenols act as antioxidants. They protect cells and body chemicals against damage caused by free radicals, reactive atoms that contribute to tissue damage in the body. For example, when Low Density Lipo-protein (LDL) cholesterol is oxidized, it can become glued to arteries and cause coronary heart disease. And it can also block the action of enzymes that cancer need for the growth and they can deactivate substances that promote the growth of cancers. The polyphenols is associated with cancer prevention is epigallocatechin-3-gallate

**Figure 7: Total Polyphenols in Amla, Gardenia and Blueberry on Activity of Polyphenols**

(EGCG). Polyphenols bind with non-heme iron in-vitro in model systems, possibly reducing its absorption. In this, Amla has more polyphenolic content when compare to Gardenia and Blueberry (Figure 7).

The use of medicinal plants extracts is intensifying world wide hunting for bioactive molecules in recent years. The present study fulfills the contention that traditional medicines are invaluable source of natural pharmaceuticals. In this regard, our investigation evidences that all the three plant extracts exhibited significant antioxidant activity. Amla and Gardenia were found to be good inhibitors of PLA and MAO-A enzymes which are involved in anti-inflammatory and depression, respectively. The Amla, Gardenia and Blueberry exhibited concentration dependant antioxidant activity. It is further suggested to screen active principles of these plant extracts and study mode of action and further to evaluate their efficacy as potential antioxidants through clinical trials.

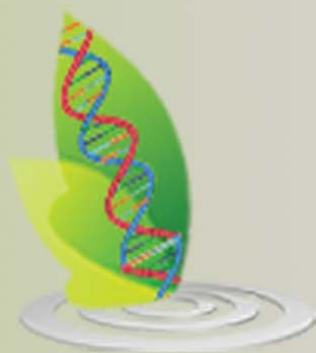
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