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INVITRO ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF THE PLANT SARCOSTEMMA BREVISTIGMA W. & A.

Krishnamoorthi Vijayalakshmi

Department of Biotechnology, Bharath Institute of Higher Education and Research, Selaiyur, Chennai-73

Chinnadurai Immanuel Selvaraj

Department of Biotechnology, School of Biosciences and technology, VIT University, Tamil Nadu, India, Vellore – 632014.

Abstract

This review was directed to explore the invitro cancer prevention agent movement of the methanolic concentrate of plant Sarcostemma brevistigma. The free extremist rummaging action of concentrate was estimated by DPPH, nitric oxide searching, superoxide searching and the cell reinforcement potential were contrasted and business cancer prevention agents, for example, nutrient C and L-ascorbic corrosive. The cancer prevention agent action of the test plant showed a portion subordinate increment when compared to business cell reinforcements. The critical free extremist scavenging movement of S. brevistigma may be ascribed to establish substance of carotenoids, free phenols and unsaturated fats.

Keywords: Sarcostemma brevistigma, DPPH, vitamin C, Carotenoids, Superoxide radical

Introduction

Sarcostemma brevistigma, Wight & Arn. from Asclepiadaceae. It is widely known as Somakalli and Kodikalli It is used for several ailments in traditional systems of medicine. It is hot, evergreen, bitter, tonic, expectorant, pungent, dry, woody creeper in nature and found throughout India. The decoction is used for throat and mouth infection. Roots (Fresh) are prescribed for jaundice[1,2]. The plant is used for several purpose such as diuretic, laxative, aphrodisiac, anthelmintic, leukoderma, bronchitis and hepatoprotective activity[3]. The juice is suggested for symptoms like pain in muscles, gleet, gonorrhoea and also given as an astringent to children[4]. This plant leaves can stimulate articulatory system, and increases urination[5]. In Tamil Nadu, the leaves, roots, and latex of the plant are

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employed in treating rheumatism, bronchitis, asthma, arthritis, chronic ulcer, skin diseases[6], ear ache, dog bite, bone fracture, snake bite, dysentery, leprosy, tumor, constipation, fever and cough (alkaloids and glycosides are responsible)[7].

In a biological system, generation of toxic oxidants and further development of oxidative stress (by gradual increase of free radicals) commonly occurs due or leads to any disease factor. Diabetes and its associated metabolic disorder of liver damage, cardiovascular disease, inflammation and autoimmune disease (Arthritis) are some diseases caused mainly due to oxidative stress and damage[8,9]. Oxidative pressure is an essential etiological element to the pathophysiology of assortment of degenerative or neurotic conditions like maturing, malignant growth, coronary illness, Alzhemier's sickness, atherosclerosis and inflammation[10].

Oxidative stress associated with type-II diabetes generate free oxygen radicals which initiate peroxidation of lipids, that stimulates inactivation of enzymes, glycation of protein and alterations in the structure and function of collagen basement and other membranes[11]. Active phagocytes like neutrophils, eosinophils, monocots and macrophages can generate large amounts of superoxide to act against foreign organisms. But in chronic inflammation, this protective mechanism may itself be damage. A one more fundamental physiological free extremist made by vascular endothelium as a loosening up factor is nitric oxide, yet on the off chance that it is unreasonable it may respond as harmful. Superoxide along with iron and copper ions can form hydroxyl radical or by combining with nitric oxide gives peroxynitrite. It decomposes to toxic products including hydroxyl radical, nitrogen dioxide gas and nitronium ion. The control of these toxic oxidants is very important by intercepting any of that generated and inactivation by blocking the propagation of chain reaction is the characteristic of an antioxidant compounds. Antioxidants are the compounds of exogenous or endogenous in nature which neutralise the possibly harming activity of free revolutionaries of such shaky particles as peroxyl revolutionary, hydroxyl extremist, singlet oxygen and peroxy nitrate radicals[12].

A strong cancer prevention agent compound is the one which end the dangerous impact of free extremists in cells and limits the tissue or cell harm by poisonous metabolites.. Antioxidants can delay to prevent the oxidation of cellular oxidizable substrates to preserve food quality from oxidative deterioration of lipids. It is possibe by scavenging ROS, activating detoxifying proteins, or by preventing the generation of ROS.

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The antioxidant compound may be synthetic or natural, but synthetic are liable to liver damage and carcinogenesis[13]. Generally regular cell reinforcement intensifies follow up on lipid free extremists and break the chain eg. Phytochemicals[14], [15], vitamins, mineral antioxidants[16] etc. Flavonoids are the most well-known phytochemicals present in numerous food varieties and restorative plants in shifting sums. It is also known to exert potent antioxidant activity against superoxide radical[17] by its redox properties and quenching of singlet oxygen.

In modern years there is a rise in the areas related to newer developments in prevention of disease especially caused by the role of free radicals and antioxidants. So it will be relevant to examine the possible role of 'free radicals' in disease and antioxidants in its prevention[18]. Plants have automatic antioxidant molecules, such as ascorbate and glutathione (GSH), as well as secondary metabolites including carotenoids (α-and β carotene, fucoxanthin, astaxanthin), mycosporine-like amino acids (mycosporine-glycine), catechins (e.g., catechin, epigallocatechin, gallate, pholorotnnins, eckol and tocopherols (α-,χ-δ-tocopherols). Some of the compounds from *Sarcostemma brevistigma* were analysed through GC- mass spectrometry and are reported to have antioxidant activity are Butyl glycol acetate, Methyl 2-diazo-3-oxo-4-propylhept- 6-enoatean ester, O, O-dipropyl isopropylphosphonate, Tricarbonyl[ü(4)-diethyl-2,5-dihydro-2,3-dimethyl-exo -2-phenyl-1H-1,2,5 azasilaborol], iron and 6á-Acetylamido-5à-hydroxyandrostane-3á,7á-diiacetate[19]. The presence of bergenin, brevine, brevinine, sarcogenin, sarcobiose were noticed by phytochemical studies while functional groups were confirmed by FTIR analysis as follows; organic halogens, carboxylic acids, anhydrides, carbohydrate, alcohols, phenols, esters, amines, ethers, amides, glycosides, nitrates, nitriles and sulphur derivatives[20].

Multiple mechanism of enzymatic and non-enzymatic antioxidant systems present in human body can protect the cellular molecules against reactive oxygen species (ROS) inducing damage. The mammalian cell defences to protect nuclear DNA from the oxidants, by categorized it away from mitochondria and peroxisomes and also by surrounding the non replicating nuclear DNA with histones and polyamines[21]. The current review was directed to assess the free revolutionary rummaging movement of methanolic concentrate of *Sarcostemma brevistigma* as there is no much logical approval for this species on its restorative employments.

Materials and methods

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DPPH Scavenging Activity

DPPH quenching ability of extracts was measured accordingly[22]. The methanol DPPH solution (0.15%) was mixed with serial dilutions (200–1000 µg/ml) of the extracts and after 10 min, the absorbance was read at 515 nm. The antiradical activity was expressed as IC_{50} (µg/ml), (the antiradical dose required to cause a 50% inhibition). Vitamin C was used as standard. The ability to scavenge the DPPH radical was calculated using the following equation:

Where A0 is the absorbance of the control at 30 min, and A1 is the absorbance of the sample at 30 min. All samples were analysed in triplicate.

$$\text{DPPH scavenging effect } \% = \frac{A0 - A1}{A0} X100$$

Nitric Oxide Radical Inhibition Assay

Sodium nitroprusside in a watery arrangement at physiological pH precipitously creates nitric oxide; it interfaces with oxygen to deliver nitrite particles, which can be assessed by the utilization of Griess Illosvoy reaction23. In the current examination, Griess Illosvoy reagent was adjusted utilizing naphthylethylenediaminedihydrochloride (0.1% w/v) rather than 1-naphthylamine (5%). The response combination (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate cradle saline (0.5ml) and distinctive convergence of concentrates (200–1000 µg/ml) or standard arrangement (0.5ml) were brooded at 25ºC for 150 min. Later brooding, 0.5 ml of the response combination containing nitrite was pipetted and blended in with 1 ml of sulphanilic corrosive reagent (0.33% in 20% frigid acidic corrosive) and permitted to represent 5min for finishing diazotization. Then, at that point1ml of naphthylethylenediaminedihydrochloride (1%) was added, mixed and allowed to address 30 min.

A pink concealed chromophore was molded in diffused light. The absorbance of these courses of action was considered at 540nm against the looking at clear. Supplement C was used as certain control. The scrounging not set in stone using the formula.

$$\text{Nitric oxide scavenging effect } \% = \frac{A0 - A1}{A0} X100$$

Where A0 is the absorbance of the control at 30min, and A1 is the absorbance of the sample at 30 min. All samples were analysed in triplicate.

Superoxide Scavenging Activity

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Superoxide rummaging exercises of concentrates was controlled by checking the opposition of those with NBT for the superoxide anion created by the PMS–NADH system24. Superoxide revolutionaries were produced in 1 ml of 20 mM Tris–HCl cushion pH 8.0 containing 0.05mM nitrobluetetrazolium(NBT), 0.01mM phenazinemethosulphate (PMS) also unique grouping of concentrates (200–1000 µg/ml) were pre-hatched for 2min. The reaction was acknowledged by the expansion of 0.078 mM NADH. Bluechromogen, shaped because of NBT decrease was perused at 560 nm. Results were enunciated as 100th 100th of save of superoxide revolutionaries. Nutrient C was utilized as a positive control. The searching movement was determined utilizing the recipe.

$$\text{Super oxide scavenging effect \%} = \frac{A0 - A1}{A0} X100$$

A0 - absorbance of the control at 30 min and A1 - absorbance of the sample at 30 min. All samples were analysed in triplicate

RESULTS AND DISCUSSION

In the present study, the free radical scavenging activities of methanolic extract from the experimental plant *Sarcostemma brevistigma* were carried out using DPPH, Nitric oxide radical and Super oxide radical.

DPPH Free Radical Scavenging Activity

The reducing power of plant extract was increased by increasing its concentration, which serves as a indicator for its efficient antioxidant activity. DPPH test is a substrate describes the antioxidative activity of antioxidants. It depends on the reduction of alcoholic stable DPPH solution to non-radical form DPPH-H [yellow-coloured diphenyl picrylhydrazine] in presence of hydrogen donating antioxidant. The results showed that *S. brevistigma* at the concentration 1000µg/mL had the slightly lesser DPPH scavenging activity of 81.83 % (Table. 1and Fig. 1) when compared to the standard vitamin C which showed 97.85%. The significant P value (0.0452) is given by statistical analysis using paired T test. The result is indicative of the hydrogen donating ability of *S. brevistigma*, since the effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. The results of the current was compared to previous report[25,26] and found that, the plant contains higher amounts of polyphenols and DPPH radical scavenging activity.

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Nitric Oxide Radical Scavenging Activity

In animal body, nitric oxide plays an essential role in various types of inflammatory processes. Nitric oxide revolutionary hindrance showed that the concentrate was an intense scrounger of nitric oxide. The concentrate repressed nitrite development by testing the

Oxygen to respond with nitric oxide straightforwardly and furthermore by inhibiting its synthesis. Nitric oxide is generated from amino acid L-arginine by vascular endothelial cells, phagocytes and certain cells in the brain. It renders inactivation of impurities in the mixture of nitric oxide encounter with oxygen and produces nitric oxide. By PMS/NADH coupling reaction, the superoxide anion resultant from dissolved oxygen reduces NBT in the PMS–NADH–NBT system. Nitric oxide radical scavenging activity showed a maximum percentage of 67.69 at the concentration 1000µg/mL (Table. 2 and Fig. 2) for *Sarcostemma brevistigma* when compared to standard vitamin C which showed a slightly higher percentage of 76.58 at a concentration of 1000µg/mL. The statistical analysis described its P value (0.0275) as significant by Paired T test. The results of the present study are similar to who reported that plants and seaweeds showed significantly higher phenolic content and antioxidant activities.

Superoxide Radical Scavenging Activity:

When the body makes oxygen radical, the unpaired electron situated on oxygen is described as superoxide. Superoxide anions are the major general complimentary radicals *in vivo* and are generated in a variety of biological systems and the concentration of superoxide anion increases under conditions of oxidative stress. The enzyme, superoxide dismutase (SOD) found in mitochondria and cytosol involves in converting superoxide to hydrogen peroxide (H_2O_2) while catalases available in peroxisomes can remove hydrogen peroxide and peroxisomal oxidase producing peroxide. Likewise, an assay was carried out to test whether the methanolic extract of *Sarcostemma brevistigma* and scavenge superoxide anions. The results showed that *S.brevistigma* at the concentration 1000µg/mL had the more or less superoxide scavenging activity of 56.77% (Table.3and Fig.3). Further they are statistically analysed and P value (0.0114) were identified as significant by paired T test. The results of the present investigation were compared with previous report[27]. Decrease in absorbance was occurred due to the utilization of created superoxide anion. Superoxide is a one-electron decreased type of sub-atomic oxygen. It is an antecedent of

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different ROS (hydrogen peroxide, hydroxyl extremist and singlet oxygen) that harms tissue by responding with natural macromolecules. Accordingly, concentrate still up in the air as a powerful scrounger of superoxide extremists in a portion subordinate way. The present study also exhibited better superoxide anion inhibitory effect, in the methanolic extract of *S. brevistigma* and it can be used as an application in natural antioxidant source.

CONCLUSION

The results of the present study indicated that the methanolic extract from the experimental plant *Sarcostemma brevistigma* had better radical scavenging activity against DPPH, superoxide and nitric oxide radicals. Hence forth the plant *Sarcostemma brevistigma* may be used as good source of natural antioxidants. In addition, sustained hyperglycemia increases reactive oxygen species generation in diabetes. Streptozotocin induces diabetes by the destruction of beta cells leads to increases in the generation of ROS thus cause tissue damage. It can be overcome by SOD, catalase and other hydroxyl radical (antioxidant enzymes) scavengers. So utilizing this antioxidant properties, the plant can be used to treat oxidative stress mediated diabetes.

Table 1 Effect of methanolic extract of *Sarcostemma brevistigma* on DPPH (Free radical scavenging activity (inhibition %)

Concentration of Sample (µg)	Control	STD	Sample	% of Inhibition	
				STD	Sample
250	0.512	0.131	0.25	74.41406	51.17188
500	0.512	0.105	0.159	79.49219	68.94531
750	0.512	0.088	0.109	82.8125	78.71094
1000	0.512	0.011	0.093	97.85156	81.83594

Table 2 Effect of methanolic extract of *Sarcostemma brevistigma* on Nitric oxide radical scavenging activity (inhibition %)

Concentration of	Control	STD	Sample	% of Inhibition

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Sample (µg)				STD	Sample
250	0.585	0.373	0.517	36.23932	11.62393
500	0.585	0.328	0.411	43.93162	29.74359
750	0.585	0.246	0.274	57.94872	53.16239
1000	0.585	0.137	0.189	76.5812	67.69231

Table 3 Effect of methanolic extract of *Sarcostemma brevistigma* on Super oxide radical scavenging activity (inhibition %)

Concentration of Sample (µg)	Control	STD	Sample	% of Inhibition	
				STD	Sample
250	0.192	0.112	0.147	41.66667	23.4375
500	0.192	0.073	0.108	61.97917	43.75
750	0.192	0.037	0.095	80.72917	50.52083
1000	0.192	0.012	0.083	93.75	56.77083

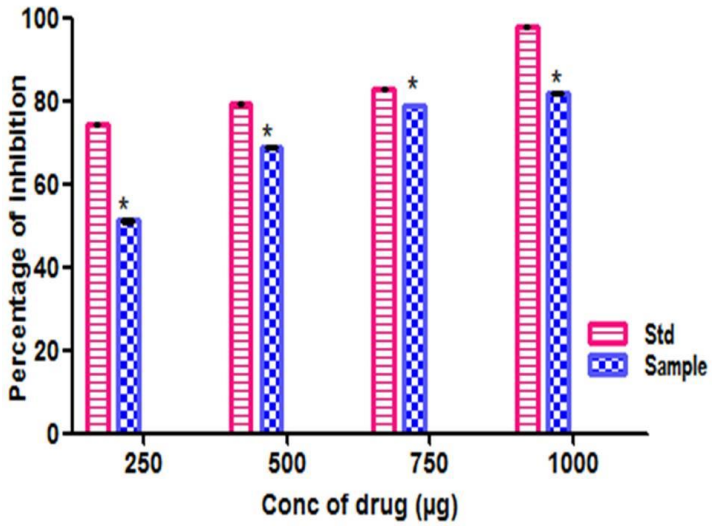

Figure 1. Effect of methanolic extract of *Sarcostemma brevistigma* (sample) on DPPH against the standard Vitamin C

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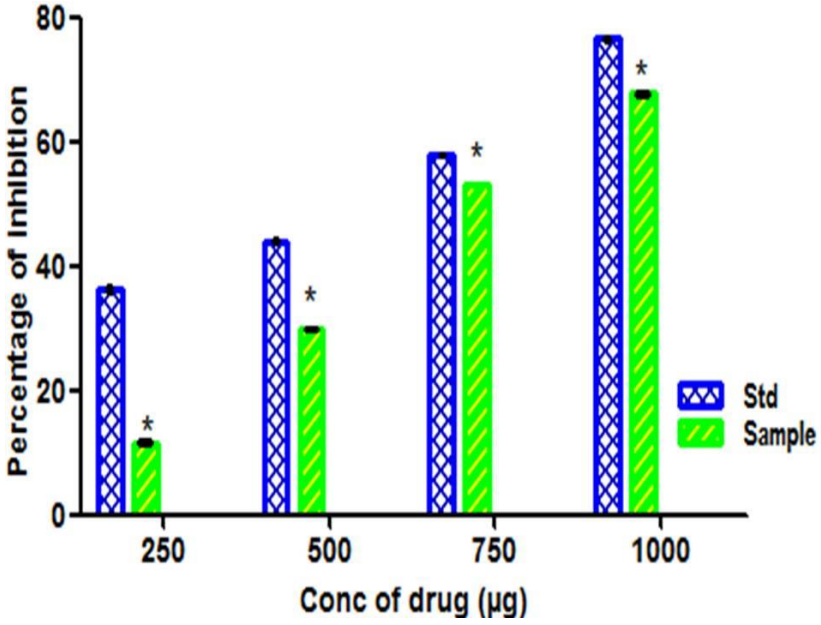

Figure 2. Effect of methanolic extract of *Sarcostemma brevistigma* (sample) on Nitric oxide radical against the standard Vitamin C

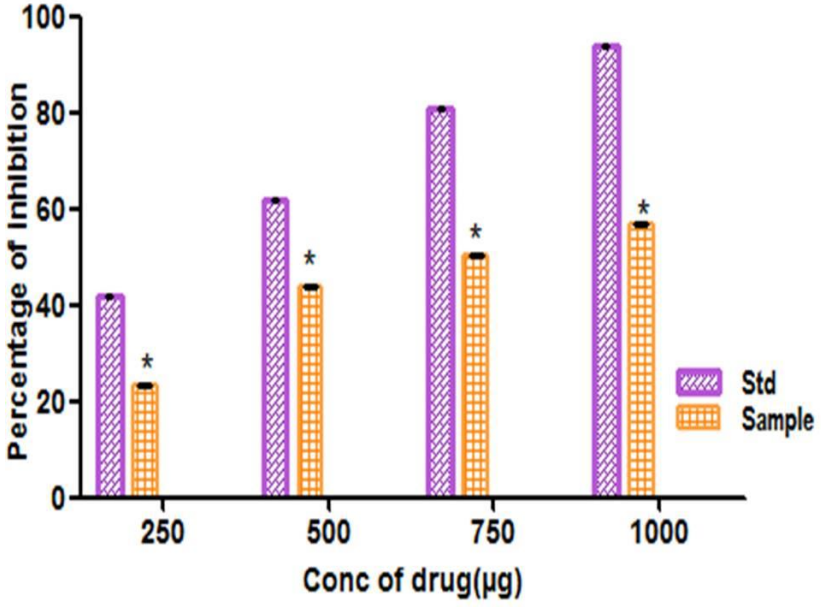

Figure 3. Effect of methanolic extract of *Sarcostemma brevistigma* (sample) on Super oxide radical against the standard Vitamin C

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