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In-vitro antioxidant potential of *Alseodaphne semecarpifolia* leaf extract

A. Charles*, M. Joseph and V. Alex Ramani

St. Joseph's College, PG & Research Department of Chemistry, Trichy, Tamil Nadu, India

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ABSTRACT

The In-vitro preliminary screening for antioxidant activity of Alseodaphane semecarpifolia leaf extract was investigated by different methods viz DPPH radical scavenging assay, Hydrogen peroxide scavenging assay and reducing power assay. The value found in DPPH method is more effective than other two methods. Thus the in-vitro studies clearly indicate that the ethanolic leaves extract of Alseodaphane semecarpifolia significant antioxidant activity and also a better source of natural antioxidant, which might be helpful in preventing the process of various oxidative stress.

Keywords: *Alseodaphne semecarpifolia*, Antioxidant activity, DPPH method.

INTRODUCTION

Alseodaphne semecarpifolia belongs from Lauraceae (Laurel family) it is commonly known as Nelthare; in Tamil, Kanaippirandai. Nelthare is a large evergreen tree up to 18 m tall, found in peninsular India. (Fig.1).

Fig. 1 Alseodaphne semecarpifolia

A.Semecarpifolia in ethno veterinary practices in India the stem bark is used for Rinderpest disease, dysentery in cattles and leach bite [1-6]. *Alseodaphne* species have been reported to contain bis-benzylisoquinolines [7] oxobis-benzylisoquinolines [8], N-Cyanomethylnorbolidine (a new aporphine alkaloid) [9], dihydroisoobtusilactone, dihydroobtusilactone, 3-epilitsenolide $D_{2,}$ 3-epilitsenolide $D_{1,}$ alseodafuranone (a lactone type compound) [10]. *Alseodaphne* species have been studied for anti-microbial [11-13], CNS stimulant and anti-inflammatory activities [14].

The ethanolic leaves extract of *A. semecarpifolia* contains various bioactive principles like flavonoids, alkaloids, phenolics etc, [15] In the present studies was to determine the *in vitro* screening of antioxidant potential of the leaf extract by following methods *viz* DPPH method, reducing power assay, Hydrogen peroxide assay *etc.*,

Free radicals are reactive species generated in the body during normal metabolic functions. These species causes cellular damage by reacting with the various biomolecules of body such as membrane lipids, nucleic acid, proteins and enzymes. This damage is the major contributor of many disorders like Cancer, Hepatopathy, Cardiovascular disorders, inflammation, diabetes mellitus, renal failure and brain dysfunction. Body has itself antioxidant system, which reacts with reactive species and neutralizes them. This natural antioxidant system includes enzymes like catalase, superoxide dismutase and glutathione, which protect the body from free radical species and prevent oxidative stress. Synthetic antioxidant like butylated hydroxyl toluene and butylated hydroxyl anisole are carcinogenic in nature. So there arises a need for natural antioxidant [16].

MATERIALS AND METHODS

The stem bark and leaves of *Alseodaphane semecarpifolia* were collected from the evergreen forests, Kolli hills, Eastern Ghats of Tamil Nadu, India. They were identified and authenticated by the Raphient herbarium of St.Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India.

Prepration of plant extract

Fresh leaves were collected, shade dried and powdered mechanically. About 100 g of the powder were extracted with 1000 ml of 70% ethanol by hot percolation method using soxhlet extractor for 4 h. The extract obtained was evaporated at 45°C to get a semi solid mass. The yield of ethanolic extract was found to be 40%. This extract was used for further studies**.**

Drugs and Chemicals

DPPH was obtained from HiMedia laboratories Pvt.Ltd., Mumbai, India. All other chemicals used in this study were of analytical grade.

DPPH Radical scavenging activity

To determine the DPPH assay of Sample by Gyamfi *et al*., Method, 2002. [17]. This method free radical scavenging potential of A.semecarphifolia leaf extracts was tested against a methanolic solution of DPPH (α, α- diphenyl - β - picryl hydrazyl) antioxidants react with DPPH and convert it to α, α-diphenyl- β - picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant activity. The change in absorbance of the samples was measured. Free radical scavenging activity was expressed as the inhibition percentage calculated using the formula.

Calculation

Percentage of anti - radical activity = [A - B/A] x 100

Where, 'A' is absorbance of control & 'B' is absorbance of sample.

Reducing power assay

To determine the reducing power assay of Sample by Yildrim et al., Method, 2001. [18] 1 ml of leaf extract was mixed with phosphate buffer (2.5 ml 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and Ferricchloride (0.5ml, 0.1%) and absorbance measured at 700nm. Increased absorbance of the reaction mixture indicates stronger reducing power. The activity was compaired with ascorbic acid standard.

Calculation

$$\text{Percentage scavenging activity} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of the control. A_{test} is the absorbance in the presence of the sample.

Hydrogen peroxide scavenging activity

To determine the Hydrogen Peroxide assay of Sample by Umamaheswari and Chatterjee Method, 2008. [19] Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). Different concentration of the extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The inhibition was calculated. Ascorbic acid was used as standard. [20-22]

Calculation

$$\text{Percentage of } H_2O_2 \text{ radical scavenging activity} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of the control. A_{test} is the absorbance in the presence of the sample.

RESULTS AND DISCUSSION

Cellular damage or oxidative injury arising from free radicals or reactive oxygen species (ROS) now appears the fundamental mechanism underlying a number of human neurodegenerative disorders, Free radicals are generated through normal metabolism of drugs, environmental chemicals and other xenobiotics as well as endogenous chemicals, especially stress hormones (adrenalin and noradrenalin) Accumulated evidence suggests that ROS can be scavenged through chemoprevention utilizing natural antioxidant compounds present in foods and medicinal plants.[23] The anti-oxidant activity of A.semecarpifolia leaf extract studied on the following methods of DPPH, Hydrogen per-oxide, and reducing power scavenging activity showed the inhibition percentage is 53.0 %, 17.0 %, and 42 % respectively. The anti oxidant activity of ethanolic extracts observed higher potential in DPPH assay.

DPPH is one of the free radicals generally used for testing preliminary radical scavenging activity of a compound or a plant extract. DPPH is a free radical in an aqueous or ethanolic solution. Substance, which is perform this reaction, can be considered as antioxidants and hence radical scavengers. This is the most widely reported method for screening of antioxidant activity of many plant drugs. DPPH assay method is based on the reduction of methanolic solution of colored free radical DPPH by free radical scavenger. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 517 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. The activity is expressed as effective concentration EC50.[24-25]

CONCLUSION

From the above studies, the ethanolic extract of *A.semecarpifolia* showed of anti-oxidant activity in all models. It is concluded that the traditional plants may represent new sources of anti-oxidants with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.

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