

**IN VITRO ANTIOXIDANT STUDY OF AERIAL PARTS OF
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ABSTRACT

In the present investigation, an attempt has been made to investigate the invitro antioxidant potential of Chloroform and methanolic extract of *Dodonaea viscosa (L.) Jacq*. The nitric oxide assay method and super oxide method has been performed at different doses (10-100µg). The results of the present study shows that the chloroform extract of *Dodonaea viscosa* possess antioxidant activity through nitric oxide and super oxide scavenging activity. The preliminary phytochemical investigation indicates the presence of flavonoids and flavono glycosides. The results are found to be significant when compared with the standard ascorbic acid. Further studies are required to determine the mechanism and isolation of active constituents involved in the antioxidant activity.

KEYWORDS: *Dodonaea viscosa (L.) Jacq*, Nitric oxide method, Super oxide method, Antioxidant activity.

INTRODUCTION

Free radicals had been implicated in several human diseases e.g. Atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, ageing, inflammatory response syndrome, respiratory diseases and cancer.^[1-4] Many herbal plants contain antioxidant compounds and these compounds protect cells against the

damaging effects reactive oxygen species such as singlet oxygen, superoxide, hydroxyl radicals and peroxynitrite.^[5-6]

Dodonaea viscosa (L) Jacq belongs to the family *Sapindaceae*. It is a cosmopolitan, variable, evergreen shrub, found in south India, ascending to an altitude of 8,000 feet. Leaves 2.50 mm long, blade-oblongate or broadly to narrowly elliptical, flowers whitish to greenish-yellow, pedicel 8-15 mm long; sepals 3-4 free, 2-1.5 mm long; petals absent, stamens 7-9, filaments very short, anthers oblong, up to 3 mm long in male flowers, up to 2mm long in bisexual flowers and reduced to staminodes or completely lacking in female flowers; ovary superior, oblong in outline, flattened, 2-3 celled, strongly rudimentary in male flowers, style 2-3 lobed. Fruit are winged 2-3-winged papery capsule (15-23 mm × 18-25 mm), white or straw-colored to brown or purplish, dehiscent by splitting along -3 central septa, each cell 2-seeded. Seeds are subglobules, more or less compressed, 3 mm in diameter, black. Seedling formed with epigeal germination. It is distributed in south India, Coimbatore, Madurai, Tiruchirappalli and Tirunelveli districts. The phytochemical studies revealed the presence of flavonoids. The plant is traditionally used as Anti-Diabetic, Anti-Oxidant, Anti-Cancer, Anti-Bacterial, Anti-Toxic, Anti-Inflammatory, Anti-Pyretic. No systematic studies on Antioxidant activity have been reported on *Dodonaea viscosa (L.) Jacq*. Hence efforts have been made to establish the Antioxidant activity.

MATERIALS AND METHODS

The aerial parts of *Dodonaea viscosa* was collected from Tirunelveli district and authenticated by **Dr.G.Johnsi Christobel., Ph.D.** Head of the Department & Research center, Department of Botany, Marthandam-629 165, Kanyakumari District. A voucher specimen of *Dodonaea viscosa* (JKKM/POC/CC-285) was deposited in the department of pharmaceutical chemistry in JKKMMRF'S - Annai Sampoorani Ammal College of Pharmacy, Komarapalyam for future reference. The air dried aerial parts of the plant material were dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh and then stored in an air tight and light resistant container for further use.

Preparation of Extract

Coarsely powdered plant material was first extracted with chloroform for 72 hours. The extract was concentrated using rotary evaporator to get solid residue. The marc left was removed, dried and successively extracted with methanol by hot percolation until complete

extraction was effected. It was then concentrated under reduced pressure and finally dried in desiccators. All the extracts were used for antioxidant studies.

Nitric oxide radical scavenging activity

Principle

Nitric oxide radical activity was done according to the method reported by Garrat *et al.*, 1964. Nitric oxide (NO \cdot) has been involved in a variety of biological functions, including neural transmission, vascular homeostasis, anti-microbial and anti-tumor activities. Despite of the possible beneficial effects of NO \cdot its contribution to oxidative damage is also reported. This is due to the fact that NO \cdot can react with superoxide to form the peroxy nitrite anion, which is a potential oxidant that can decompose to produce OH acid and NO \cdot . This procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO \cdot Which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitric ions. Large amount of NO \cdot may lead to tissue damage (Ebrahimzadeh M.A.,2010).

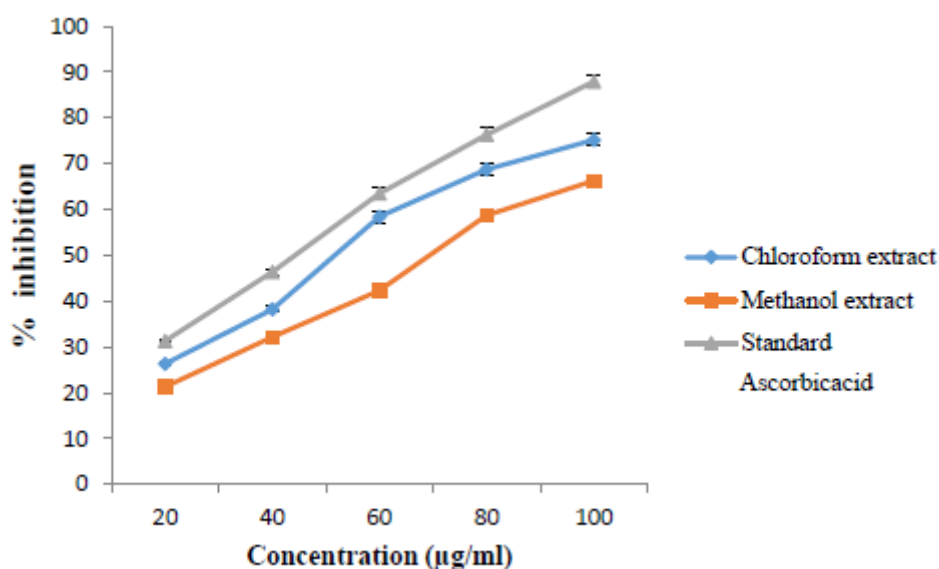
The chloroform and methanol extracts were subjected to nitric oxide scavenging activity. Sodium nitroprusside (5mmolL⁻¹) in phosphate buffered saline pH 7.4 was mixed with different concentration of the extract (20 to 100 μ g/ml) prepared in methanol and incubated at 25⁰C for 30minutes. A control without the test compound, but an equivalent amount of methanol was taken after 30 minutes, 1.5ml of the incubated solution was removed and diluted with 1.5ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthyl ethylene diamine dihydrochloride). Absorbance of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with N-1-naphthyl ethylene diamine dihydrochloride and incubated at the room temperature for 5 minutes. The absorbance of the mixture at 546nm with the spectrophotometer. Ascorbic acid was used as a standard. The percentage inhibition was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of the test}}{\text{Absorbance of control}} \times 100$$

Effects of various extracts of *Dodonaea viscosa (L.) Jacq* on Nitric oxide scavenging activity

Concentration (µg/ml)	% of activity (±SEM)		
	Chloroform extract (CETS) (µg/ml)	Methanol extract (METS) (µg/ml)	Standard (Ascorbic acid) (µg/ml)
20	26.28 ± 0.54	21.26 ± 0.36	31.27 ± 0.26
40	38.16 ± 0.65	32.16 ± 0.51	46.31 ± 0.57
60	58.41 ± 1.26	42.26 ± 0.68	63.47 ± 1.21
80	68.72 ± 1.24	58.72 ± 1.07	76.28 ± 1.46
100	75.17 ± 1.25	66.27 ± 1.25	87.91 ± 1.26

All the values are expressed as mean ±SEM (n=3) for three determinations.



Graph No. 1: Effects of various extracts of *Dodonaea viscosa (L.) Jacq* by Nitric oxide scavenging activity.

Superoxide radical Scavenging Activity

Principle

Superoxide radical scavenging activity is generally based on the anion radical which is associated with PMSNADH system. The measurement of superoxide scavenging activity is based on method as described by Liu *et al.*, with slight modifications. They are generated within PMSNADH system by the oxidation of NADH and are assayed by the reduction of Nitro blue tetrazolium (NBT).PO₄ buffer (100µM, pH 7.4) containing 1 ml NBT (156µM) solution, 1ml NADH (468µM) solution on and a sample solution of extract (20-100µg/ml) in methanol were mixed.

The chloroform and methanol extracts were subjected to superoxide free radical scavenging activity. The reaction was started when 0.1ml of phenazine methosulfate (PMS) solution

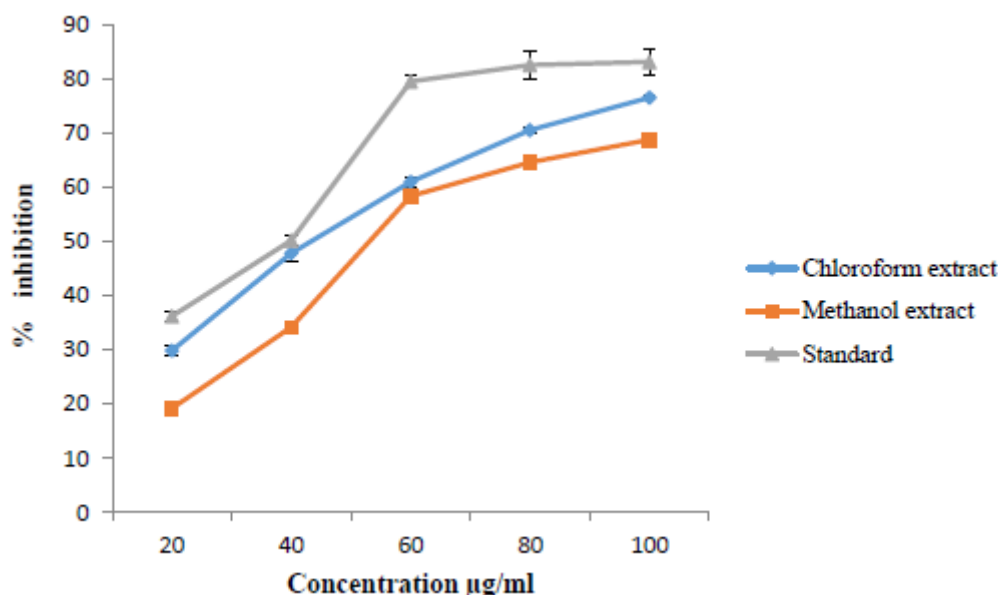
(60 μ M) was added to the mixture. The reaction mixture was incubated at 25⁰ C for 5 min, and the absorbance was read at 560nm against the corresponding blank samples. Ascorbic acid was used as a reference drug. Decreased absorbance of the reaction mixture indicated increased superoxide radical scavenging activity. The Percentage inhibition was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of the test}}{\text{Absorbance of control}} \times 100$$

Effects of various extracts of *Dodonaea viscosa (L.) Jacq* on Superoxide scavenging activity

Concentration (μ g/ml)	% of activity (\pm SEM)		
	Chloroform extract (CETS) (μ g/ml)	Methanol extract (METS) (μ g/ml)	Standard (Ascorbic acid) (μ g/ml)
20	29.94 \pm 1.03	19.26 \pm 0.91	36.28 \pm 1.04
40	47.90 \pm 1.38	34.26 \pm 0.96	50.27 \pm 1.05
60	61.07 \pm 1.03	58.45 \pm 1.21	79.64 \pm 1.03
80	70.65 \pm 0.69	64.67 \pm 1.38	82.63 \pm 2.41
100	76.64 \pm 0.34	68.86 \pm 0.69	83.23 \pm 2.41

All the values are expressed as mean \pm SEM (n=3) for three determinations



Graph No. 2: Effects of various extracts of *Dodonaea viscosa (L.) Jacq* by Super oxide radical scavenging activity.

RESULTS AND DISCUSSION

The antioxidant activity was performed for both the chloroform and methanolic extracts by nitric oxide scavenging activity and superoxide free radical scavenging activity. The chloroform extract showed maximum activity with inhibition of 75.17% Nitric oxide scavenging radical and 76.64% Superoxide scavenging radical when compared to standard ascorbic acid. The chloroform extract has better antioxidant activity than methanolic extract for all the two invitro antioxidant activity screened.

CONCLUSION

Therefore, the study may be concluded that, the aerial portion of *Dodonaea viscosa* can be used for antioxidant and anticancer activity studies, can be utilized therapeutically as a main ingredient for anticancer herbal preparations after proper formulation.

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