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Invitro Antioxidant and Antibacterial Effect of *Dodonaea Viscosa* Linn

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Background and Objectives

Free radicals are the exceptionally responsive substances associated with the pathophysiology of different illnesses like cancer, inflammation. Because of this there is a need to investigate substances with free radical inhibitory potential or antioxidant effects. The main objective of the investigation is to investigate the invitro antioxidant activity of hydro alcoholic concentrate of Dodonaeaviscosa on different invitro models.

Methods

The hydroalcoholic concentrate of D.viscosa was arranged and exposed to fundamental phytochemical investigation. The invitro antioxidant activity of D.viscosa was assessed utilizing DPPH rummaging, lessening power and nitric oxide extremist searching examine. Further, the antibacterial movement of plant remove was assessed utilizing agar plate dispersion and agar well dissemination technique on different microorganisms.

Results

In this examination, hydroalcoholic concentrate of D.viscosa showed successful restraint of free revolutionaries in DPPH rummaging, decreasing force and nitric oxide searching measures with an IC50 worth of 66.42, 32.88 and 100 µg/ml separately. The concentrate at different focus showed checked antibacterial action against the microorganism.

Conclusions

Hydroalcoholic concentrate of D.viscosa is a likely wellspring of normal natural antioxidants and fills in as a successful free radical scavenger.

Keywords: Dodonaeaviscosa, Free radicals, DPPH assay, Antibacterial

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Introduction

Free radicals are profoundly receptive atoms that contain at least one unpaired electron. They either donate or take electrons from different particles trying to combine with their electrons and create more steady species. Reactive oxygen species (ROS) are subordinates of oxygen [1] and are continually delivered in the body during different metabolic exercises like aerobic respiration and by different exogenous variables [2]. A portion of the receptive oxygen species assume a positive part in phagocytosis (oxygen burst), energy creation and guideline of cell development, intra cell flagging and so forthNotwithstanding, free radicals created by daylight, UV light, ionizing radiation, synthetic responses and metabolic cycle have a wide assortment of obsessive impacts.Reactive oxygen species produced in the life form are generally taken out or killed by an effective organization of guard instrument in the body. At the point when the arrangement of these free radicals or responsive oxygen species surpasses the degrees of defencing instrument, it prompts the harm of tissues, bio atoms and further, prompting illness conditions particularly degenerative sicknesses, for example, Aging, Diabetes, Arthritis, Carcinogenesis, and Cardio Vascular infections [3-6].

Antioxidants are significant substances that assume a pivotal part in postponing, blocking, and forestalling oxidative responses catalysed by free revolutionaries and in this manner giving assurance to people [7]. Because of this uncommon capacity there is an expanded utilization of cell reinforcements for the equilibrium of receptive oxygen species. Presently a day, a large portion of the cancer prevention agents are fabricated artificially. A few engineered cell reinforcements, for example, Butylated hydroxyl anisole (BHA), Butylated hydroxyl toluene (BHT), Tertiary butylated hydroxyl quinone (TBHQ), and Gallic corrosive esters are monetarily accessible.Such engineered cancer prevention agents are known to have likely results and have some level of cancer-causing nature when taken in vivo" [8-12]. Henceforth their utilization is being confined now-a-days. “Antioxidant substances from plant materials are protected and end the activity of free extremists along these lines shielding the life form from different sicknesses. Consequently, an extraordinary interest to evaluate restorative plants for the presence of characteristic cell reinforcements has extraordinarily expanded.

Plant inferred common mixtures, for example, Flavonoids, Terpenes, Alkaloids and so on have gotten impressive consideration lately because of their pharmacological properties including Antioxidant, Antimicrobial and Anti-fiery exercises [13,14]. Dodonaeaviscosa Linn. (Sapindaceae) regularly known as 'virali' is an evergreen enduring bush generally disseminated in Western Ghats and Tamilnadu. The legends guarantee uncovers that the leaves have been utilized for the treatment of cerebral pains and spinal pains by the Muthuvan clans of the Kerala area. Boiling water decoction of leaves is utilized to decrease swellings, spinal pains and steam inward breath is utilized to reduce cold. Further, in conventional clinical practice D. viscosa is utilized to mitigate stomach torment, heaps and ulcer. Past examinations have announced the mitigating, antimicrobial, neighbourhood sedative and smooth muscle loosening up movement of D. viscosa[15, 16]. Thus, the current examination was meant to assess the cell reinforcement capability of Hydro alcoholic concentrate of D. viscosa on different invitro models.

Materials and Methods

Plant Material

The leaves of D. viscosa were gathered in the month August 2018, from Trichy, Tamilnadu, India. The plant material was recognized and confirmed by the botanist. The plant materials were dried under conceal, cut into little pieces, pounded utilizing a

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mechanical processor and went through 40 mesh sieve and put away in an impermeable compartment for additional utilization.

Extraction of Plant Material

The powdered leaves of D. viscosa were extricated with hydro alcohol at room temperature. After comprehensive extraction, the dissolvable was gathered and separated. The dissolvable was focused under lessen pressure at 50-55°C. The concentrated hydro alcohol separates were kept in desiccators for additional utilization.

Qualitative Phytochemical Analysis

The crude hydro alcoholic concentrate of D. viscosa leaves were dissected for the presence of different phytoconstituents by keeping standard phytochemical protocols. The presence of alkaloid (Dragendorff reagent, Mayer's reagent, Hager's reagent and Wagner's reagent), flavonoids (Shinoda-Paw test), steroids (Liberman Burchard test and Salkowski's response), terpenes (Vanillin sulfuric corrosive reagent) and carbs (Fehling's test and Molisch test) were analysed.

Invitro Antioxidant Activity

Invitro Antioxidant Activity by DPPH Assay

The scavenging activity for DPPH free radicals was estimated by the methodology depicted by Braca et al., 2001. An aliquot of 3 ml of 0.004% DPPH arrangement in ethanol and 0.1 ml of plant extricate at different fixations were blended. The blend was shaken enthusiastically and permitted to arrive at a consistent state at room temperature for 30 min. Decolourization of DPPH was controlled by estimating the absorbance at 517 nm. A control was arranged utilizing 0.1 ml of individual vehicle in the spot of plant extricate/ascorbic corrosive. The rate hindrance movement was determined as [(A0-A1)/A0] ×100, where A0 was the absorbance of the control, and A1 was the absorbance of the plant extract/ascorbic acid.

Reducing Power Assay

Different convergences of the plant separate in relating solvents were blended in with phosphate cushion (2.5 ml) and potassium ferricyanide (2.5 ml). This blend was kept at 50ºC in water shower for 20 minutes. Subsequent to cooling, 2.5 ml of 10% trichloro acidic corrosive was added and centrifuged at 3000 rpm for 10 min at whatever point fundamental. The upper layer of arrangement (2.5 ml) was blended in with refined water (2.5 ml) and a newly pre-arranged ferric chloride arrangement (0.5 ml). The absorbance was estimated at 700 nm. Control was set up in comparable way barring tests. Nutrient E at different fixations was utilized as standard. Expanded absorbance of the response blend demonstrates expansion in diminishing force.

Nitric Oxide Radical Scavenging Assay

Sodium nitroprusside (5 mm) in standard phosphate cushion saline (0.025 M, and pH 7.4) was brooded with different centralizations of concentrates, and cylinders were hatched at 29°C for 3 hrs. A control test without the test compounds however with a comparable measure of cradle was directed in an indistinguishable way. After 3 hrs hatched examples were weakened with 1 ml of Griess reagent. The absorbance of the shading created during diazotization of nitrite with sulphanilamide and its ensuing coupling with naphthyl ethylenediamine hydrochloride was seen at 550 nm on spectrophotometer. A similar strategy was led with ascorbic corrosive which was standard in contrast with test[8].

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Bacterial Cultures

Bacterial cultures were acquired from Microbial Type Culture Collection, Chandigarh, India. The microorganism utilized for the current examination incorporate*Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC441), *Escherichia coli* (MTCC40), *Klebsiella pneumonia* (MTCC3384), *Pseudomonas aeruginosa* (MTCC741), *Proteus mirabilis*, (MTCC425). Every one of the bacterial cultures were kept up in supplement agar and put away at 4^oC.

Preparation of Inoculum

A few settlements were moved to sterile peptone water (5 ml) from the sub refined creature. The suspensions were blended for 15 seconds to guarantee homogeneity and in this way weakened to coordinate with the turbidity of a 0.5 McFarland standard (for example OD = 0.12–0.15 at k = 530 nm, relating to 1–5 x 10^6 CFU/ml).The antimicrobial assay was performed by two strategies viz. agar plate dissemination strategy and agar well dispersion technique. Mueller Hinton agar (MHA) was set up in plates as the media for test microscopic organisms. The bacterial inoculum was spread equitably on the outside of the MHA plates using a sterilized cotton swab.

For agar disc diffusion method, sterile channel paper circles (6mm) were immersed with various convergences of the test compound, permitted to dry and presented on the upper layer of the cultivated agar plate.For agar well dissemination technique, an all-around was set up in the plates with the assistance of a plug drill (0.6cm). 100 µl of the test compound was brought into the well. The plates were hatched for the time being at 37°C For each bacterial strain controls were kept up where unadulterated solvents were utilized rather than the concentrate. Sterile refined water filled in as bad control. The outcome was gotten by estimating the zone distance across. The investigation was done threefold and the mean qualities are introduced.

Results

In this investigation, D. viscosa inspired solid hydrogen giving capacity with an IC50 worth of 66.42 µg/ml in the DPPH free revolutionary rummaging examine. The outcomes were appeared in table 1. D. viscosa inspired solid nitric oxide searching movement with an IC50 worth of 32.88 µg/ml. The outcomes were appeared in table 2. In this examination, the diminishing force of D. viscosa increments with an expansion in the focus. The D. viscosa showed most extreme movement at the centralization of 100 µg/ml.In this investigation the antibacterial movement of the plant extricates were assessed by agar plate dissemination technique and agar well dispersion strategy. The concentrate at different focus showed stamped antibacterial action against the microorganism utilized in the investigation. The outcomes were appeared in table 4 and 5.

Table 1: Invitro DPPH radical scavenging effect of *D. viscosa*

Concentration (µg/ml)	**Percentage Inhibition**	
	Vitamin C	***D. viscosa***
10	9.8 ±0.98	8.76±0.65
20	17.9±0.92	16.76± 0.87
40	34.4±0.85	32.56±0.65
80	68.7±0.92	66.54±1.12
100	83.2±1.02	79.54±1.24
IC_{50} (µg/ml)	61.01	66.42

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Table 2: Invitro nitric oxide scavenging effect of *D. viscosa*

Concentration (µg/ml)	Percentage Inhibition	
	Vitamin C	*D.viscosa*
10	40.26±0.56	31.56±0.42
20	51.46±0.76	43.65±0.62
40	64.12±0. 87	60.76±0.78
80	83.14±0.92	77.76±0.87
100	96.56± 0.95	88.21±1.21
IC_{50} (µg/ml)	12.54	32.88

Table 3: Invitro reducing power ability of *D.viscosa*extract

Concentration (µg/ml)	Absorbance	
	Vitamin E	*D.viscosa*
10	0.40±0.02	0.24±0.01
20	0.50±0.05	0.35±0.04
40	0.68±0.07	0.72±0.08
80	1.02±0.08	1.0±0.07
100	1.78±0.07	1.72±0.1

Table 4: Antibacterial activity of *D.viscosa*extract by disc diffusion method

Microorganism	*D.viscosa*extract concentration (µg/ml)			
	20	**40**	**80**	**100**
S. aureus	22	24	27	31
B. subtilis	15	18	22	26
E. coli	21	24	28	33
K. pneumonia	13	16	20	24
P.aeruginosa	11	15	19	23
P. mirabilis	9	13	17	22

Table 5: Antibacterial activity of *D.viscosa*extract by well diffusion method

Microorganism	*D.viscosa*extract concentration (µg/ml)			
	20	**40**	**80**	**100**
S. aureus	21	24	28	32
B. subtilis	14	17	23	25
E. coli	19	22	25	31
K. pneumonia	11	14	18	23
P.aeruginosa	12	16	20	25
P. mirabilis	10	12	14	21

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Discussion

Free radicals orchestrate a vital role in the progression of tissue obliteration and different neurotic occasions like maturing [1], tumours, aggravation and neuro degenerative sicknesses [2]. The cancer prevention agent capability of restorative plants and its phytoconstituents which evokes free extreme extinguishing potential gets legitimate consideration for moderation of oxidative pressure and subsequently secures cells and organs [3,4].

The highly reactive DPPH extremist is regularly utilized for the assessment of cell reinforcement has been utilized generally for the assurance of essential cancer prevention agent potential. DPPH is the compound which has proton free extremist with explicit retention territory and diminishes successfully when uncovered proton free extreme inhibitors. The DPPH response is observed by absorbance decline at 517nm by the cell reinforcement compounds. The DPPH hindrance measure is relying upon the nature of the decolourization of stable DPPH revolutionary by the activity of cell reinforcements. The response is the change of receptive DPPH extremist to 1, 1-diphenyl-2-picryl hydrazine in the presence of antioxidants.

The DPPH radical scavenging capability of antioxidants is fundamentally because of their capacity to give hydrogen. Further, the cell reinforcement capacity of restorative plants has positive connection with grouping of phenolic compound present in the concentrates. In the current investigation, D.viscosa separate showed successful restraint of DPPH free extremist, and along these lines demonstrates the response between plant concentrates and revolutionary age, which prompts revolutionary searching by giving the hydrogen particle.

Reducing power assay shows the diminishing capacity of the cancer prevention agents by giving electrons and helps the decrease of Fe^{3+} ferricyanide complex to the ferrous (Fe^{2+}) particles, and along these lines shapes a blue green hued which is estimated at 700nm and compares to the Fe^{2+} sum in the response combination. The current investigation shows the impact of D.viscosa concentrates to diminish the ferric to ferrous particles. The diminishing capability of plant separates are principally because of quality of reductions and their belongings is because of free extreme chain end by giving hydrogen molecule and furthermore responds with peroxide forerunners and decreases the peroxidation interaction [5].

Nitric oxide (NO) is an indispensable compound go between delivered from macrophages, endothelial cells, mind and controls the wide scope of organic cycle. Expanded level of NO is seen in different neurotic conditions. The age of NO in natural tissues is intervened nitric oxide synthase (NOS) which catalyses the change of arginine to citrulline and NO through five carbon metabolic oxidative pathway [7].

The delivered NO effects affects organs and adjusts its underlying and useful limit. NO rummaging potential is assessed by the decrease in absorbance at the scope of 550 nm incited by free extreme scroungers. For assessing the cell reinforcement viability of plant removes by NO rummaging the adjustment of absorbance of NO is recorded. Nitric oxide extremist is produced when it responds with oxygen or superoxide, to frame NO_2, N_2O_4, N_3O_4, NO_3 and NO_2 which are exceptionally receptive. These responsive extremists make a condition of oxidative pressure and harm the organs [8].

In the current investigation the D.viscosa remove showed compelling nitric oxide rummaging because of its cell reinforcement potential. The cancer prevention agent movement of D.viscosa is principally because of the presence of flavonoids, for example, quercetin, rutin and myricetin.Mounting examines shows that Gram positive microorganisms are more powerless against plant removes as that of the Gram-negative microbes [17,18]. This situation is because of that the cell mass of Gram-positive microbes is single layer and

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Gram-negative cell divider is complex construction [19]. Past investigations show the antibacterial movement of D.viscosa utilizing bio autography strategy [20].

Conclusion

The current examination affirms the antioxidant capability of D.viscosa plant extricate in different invitro models. Further, phytochemical and segregation contemplate are profoundly justified to discover the phytoconstituents answerable for cell reinforcement movement. Further, the investigation subsequently uncovers the viability of D.viscosa remove against pathogenic microbes causing different human diseases.

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