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Original Article

Anti-neoplastic activities of targeted bio- moieties of *Dodonaea Viscosa*

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ABSTRACT

The objective of the present study was focused phytochemical screening of *Dodonia viscosa* (DV) Versatile medicinal plant. The arial parts of DV was subjected to the super critical fluid extraction (SCFE) and confirmed for the presence of many phytoconstituents like flavonoids, glycosides, saponins etc. Further the procured extract was subjected to column chromatography and isolated the active bio-compounds, those were subjected for antioxidant activity by following DPPH and SOD method. Antineoplastic activity was carried out against Erlichs Ascites Carcinoma (EAC)-induced mice. The extract and isolated bio compound showed good antioxidant and anticancer activity, however further investigations of MTT-assay is required for structural elucidation of bioactive compounds.

Key words: SCFC, EAC**,** *Dodonaeaviscose*, DPPH, SOD

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INTRODUCTION

Among various diseases, cancer has become a big threat to human beings globally. As per Indian population census data, the rate of mortality due to cancer in India was high and alarming with about 806000 existing cases by the end of the last century. Cancer is the second most common disease in India responsible for maximum mortality with about 0.3 million deaths per year[1-6]. This is owing to the poor availability of prevention, diagnosis and treatment of the disease. All types of cancers have been reported in Indian population including the cancers of skin, lungs, breast, rectum, stomach, prostate, liver, cervix, esophagus, bladder, blood, mouth etc. The causes of such high incidence rates of these cancers may be both internal (genetic, mutations, hormonal, poor immune conditions) and external or environmental factors (food habits, industrialization, over growth of population, social etc.). In view of these facts, the advent of modern targeted synthetic/natural compounds has undeniably improved cancer patients. Many molecules that will approaches for the discovery of Novel active derivatives are inhibited the carcinogenesis and they possess chemo preventive properties. The *Dodonaeaviscose* is normal shrub with green leaves and widely distributed in India. The various parts of this plant have excellent medicinal applications such as antimicrobial, cardio-vascular disorders, analgesic, anti-inflammatory actions[7-10]and neurological disorders[11]. Based on this medicinal property of the plant, the present research was targeted that *Dodonaeaviscose* (DV) of aerial parts are extracted by modern sophisticated extraction procedure called Super Critical Fluid extraction (SCFC) and isolated fractions were subjected to antioxidant and anti-neoplastic activities against Ehrlichs Ascites Carcinoma (EAC).

MATERIALS AND METHODS

Collection and Authentication:

*Dodonaeaviscosa*aerial are collected from rural areas of Davangere district and they were authenticated by the taxonomist. The collected plant materials are washed and subjected to shade drying for a week further the plant material is blended for supercritical fluid extraction.

Supercritical Fluids: A fluid at supercritical condition, also referred to as a dense gas, is a fluid above its critical temperature (T_C) and critical pressure (P_C) to a certain extent: to be supercritical, the reduced temperature Tr (i.e. T/T_C) must not exceed 1.2 or 1.3, whereas the reduced pressure Pr (i.e. P/P_C) may be as high as allowed by technological limits.

At suitable conditions, any fluid can reach its supercritical state. However, only those having a critical temperature not far from ambient temperature can be used as alternative solvents for the extraction of MAPs. Carbon dioxide (CO_2), with T_C=31.06° C and P_C=73.81 bar, is the most attractive solvent, because of its proprieties regarding toxicity, flammability and cost. The possibility of using supercritical fluids (SFs) as extraction solvents is directly linked to their density. In fact, according to an empirical correlation proposed by Chrastil in 1982,

$S = \rho a \exp(bT + c)$

where,

S is the solute solubility,

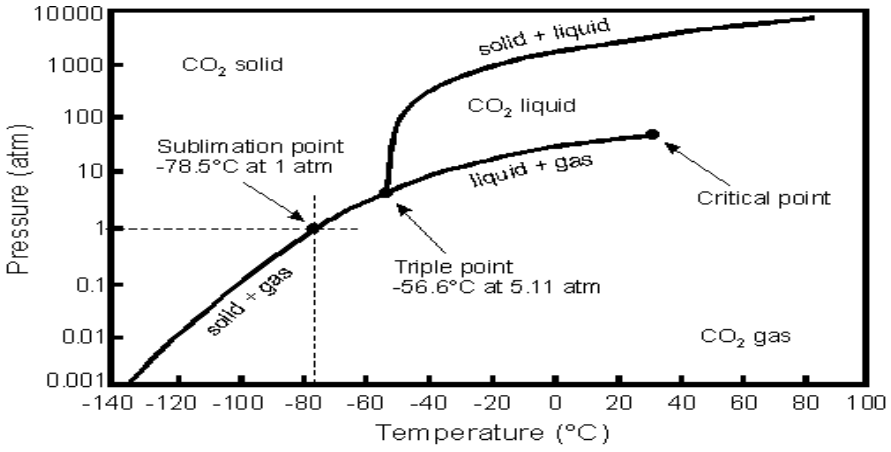
ρ is the solvent density

T is the absolute temperature

a, b and c are correlation parameters to be adjusted to experimental solubility data in supercritical CO_2

When a fluid approaches the critical conditions, its density gets closer and closer to that of the liquid state. This can be seen, for CO_2, in Figure 2.1, where density isotherms are plotted against the reduced pressure.

For example, at T = 35° C and P = 200 bar, ρ = 866 kg/m3.



Pressure-Temperature phase diagram for CO_2.

Apparatus: The apparatus used for extraction is SFT-110. Safety precautions and operating measures were followed during the running of equipment which includes testing of pressure range to be operated and leakage tests for supercritical fluid. The Figure 3.2 indicates the block diagram of the apparatus.

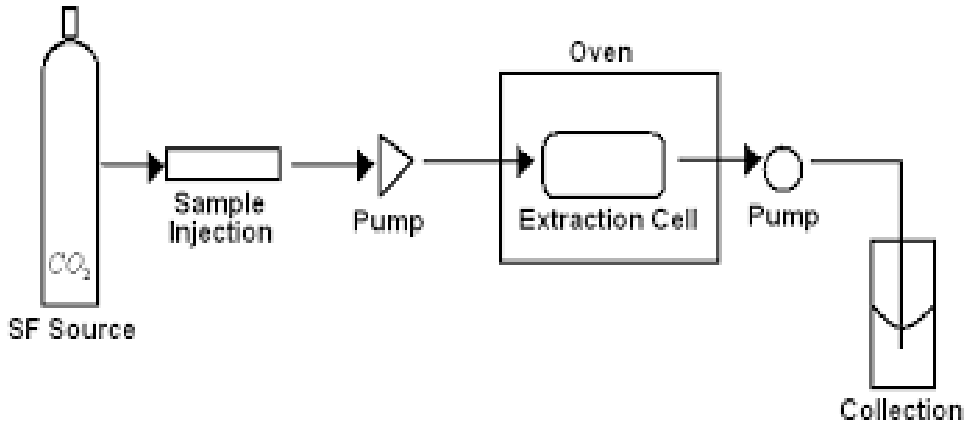


Figure1. Schematic diagram of Supercritical extraction set up.

SFT-110 Series System Specifications
3.3.1 Standard Configuration
Temperature and Pressure Display: Independent LED displays.
Temperature Range: Ambient to 200°C.
Temperature Precision: +/- 0.5°C.

Operating Pressure: 10,000 psi upper pressure limit. Front keypad control, with LED display.
Constant pressure mode of operation. Flow Rates: 0.01 – 24.00 ml/min liquid CO2 (+/- 2% accuracy).Over Pressure Safeguard: High / Low pressure alarms and rupture disc assembly. SFT-10 High Pressure Pump: Dual aluminium heads, furon seals and sapphire pistons, integrated thermoelectric cooling, cam-driven pump mechanism with single stepper motor dive, dual ball and seat check valves (ruby ball, sapphire seat). The pump's constant pressure mode features a selectable pressure set point. Flow rate auto-adjusts to maintain pressure. Restrictor Valve: Heated up to 200°C; User selectable set point; Resistant to blockage. Preheater: Improves temperature consistency of the fluid by heating the fluid before it reaches the main pressure vessel.

Extraction Vessel: Accommodates vessels ranging in size from 5 ml to 100 ml. (Up to 500 ml for the SFT-110XW) Vessels come with 5 micron frits and are interchangeable.
SFT-110 Dimensions: Width: 29 cm, Depth: 57 cm, Height: 76 cm.
SFT-110XW Dimensions: Width: 40 cm, Depth: 57 cm, Height: 102 cm
SFT-110 Weight (without vessel): 24 kg (52 lbs)
SFT-110XW Weight (without vessel): 28 kg (60 lbs)

Experimental procedure

1. Turn on the power to the SFT-110 SFE unit.
2. Turn on the Peltier cooler at least thirty minutes prior to the start of the experiment.
3. Powder 50 grams of raw material i.e., dried *Dodonaeaviscosa*Place the powdered *Dodonaeaviscosa*material into the SFT-110 Unit's processing vessel and seal.
4. Ensure that both the static/dynamic valve and restrictor valve are closed.
5. Open the carbon dioxide tank valve to allow the carbon dioxide to come into the unit (~750 psi).
6. Set the oven temperature to 40°C and the restrictor block to 40°C.
7. Set the pressure on the pump to 2000 psi. The pump should begin to actuate to pressurize the sample vessel. This will take between 12-15 minutes.
8. Once the pressure is up to 2000 psi, allow the sample to "soak" at that pressure for 15 minutes for 40°C.
9. Open the static/dynamic valve to allow free flow of carbon dioxide through the restrictor valve. Adjust the restrictor valve to achieve about 24ml/min of liquid carbon dioxide. Flow dynamically for 15 minutes. The pump should actuate and continue to maintain sample vessel pressure.
10. Close the static/dynamic valve and allow to "soak" for an additional 15 minutes before repeating the dynamic flow step above. Repeat the static soak and dynamic flow step 5 more times.
11. Set the vessel temperature to ambient and set the pressure control down to ambient. Allow the unit to vent. When the vessel has reached ambient pressure, disconnect the inlet and outlet fittings, open the vessel and remove the *Aeglemarmelos* powder.

Similar procedure is carried out by varying the parameters such as temperature (45°C, 50°C and 55°C), pressure (2000 psi, 3000 psi and 4000 psi) and time (20, 25 and 40 minutes).

Phytochemical Investigation

Phytochemical investigation of SCFE of *Dodonaeaviscosa*was carried out the presence of activeconstituents were enlisted in the following table1.

Table 1. Phytochemical Investigation summary

Plant Material	Yield	Consistency	Phyto-constituents
SCFE-***Dodonaeaviscosa***	12gm	Dark brown oily viscous liquid	Flavonoids, steroids, saponinglycosides, coumarins, triterpenoids and charbohydrates.

Isolation and Thin line chromatography: The SCFE (12g-DV) was chromatographed over silica gel (100-200 mesh) on column 55 cm length and 6 cm diameter. Elution was carried out with solvent mixtures of increasing polarities (pet. ether:chloroform: Ethylacetate: Ethanol). Fractions were collected in 100ml portions and monitored by TLC (silica gel G as adsorbent, solvent system ethyl acetate: formic acid: glacial acetic acid: water (80: 11: 11: 47) chloroform: ethyl acetate (50:50) for DV which contains flavonoids and dioxone.

Figure 3. Column chromatography

Figure 4. Isolated fractions of DV

Antioxidant activities of *Dodonaea viscosa* by DPPH and SOD method[13-15]

Acute toxicology test: Acute toxicity study was performed using albino mice and doses were fixed as per OECD guidelineNo.423 and adopted CPCSEA protocol. During acute toxicity studies, SCFE of ***Dodonaea viscosa*** produced any abnormal effect - nor moribund stages no death was observed.

Experimental Animal: Albino mice of either sex (8-10 weeks old) weighing 20-25 g, are will be used for theexperiment. The animals are maintained under proper environmental conditions i.e,temperature 25 ± 2 ºC and humidity 50 ± 5% with a 12 h light and dark period.

1. DPPH radical scavenging method; the assay will be carried out in 96 well microtitre plate.

2. Estimation of super oxide dismutase (SOD) by nitro blue tetrazoline (NBT)method; SOD in the serum was estimated using methods reported in the literature.3. *In vitro* antioxidant activity; Healthy Swiss albino mice were divided into 5groups (n=6), the Group-I serves as control, and Group II receives ascorbic acidas standard and III, receives ethanolic extract and Group IV receives chloroformextract and V serve as blank. The treatment is given up to 8 days and 9thday micewill be anaesthetized using diethyl ether and blood will be collected retro orbital/heart and kept at 37 ˚C in the incubator for 30 min. Later subjected to centrifugeat 2000 rpm for 15 min, to get clear supernatant serum from EAC-carcinoma was induced mice.

Table 2. Antioxidant activities of *Dodonaea viscosa* seeds by DPPH method

Sl.No.	Treatment	Aborbance at 515nm	Percentage of Scavanging
1	Control	0.347	0
2	Standard	0.122	96
3	Ethanol		
		0.333	3.86
		0.064	81.46
		0.066	80.86
4	Chloroform		
		0.580	-67.17
		0.313	9.79
		0.155	55.27
5	DVF1		
		0.147	57.40
		0.264	23.86
		0.308	11.21
6	DVF2		
		0.367	-6.0
		0.063	81.72
		0.536	84.55

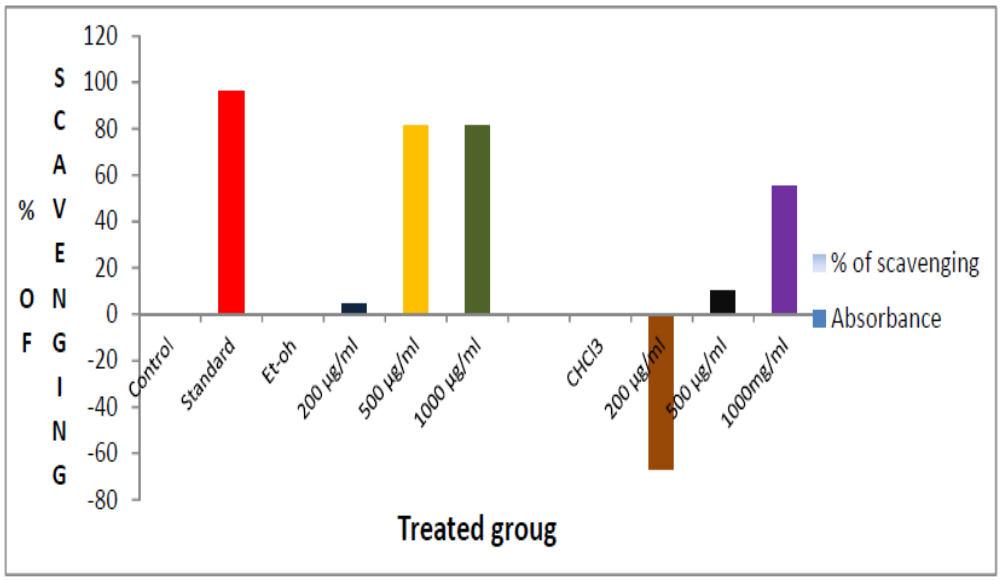


Figure 4. Estimation of DPPH in treated group

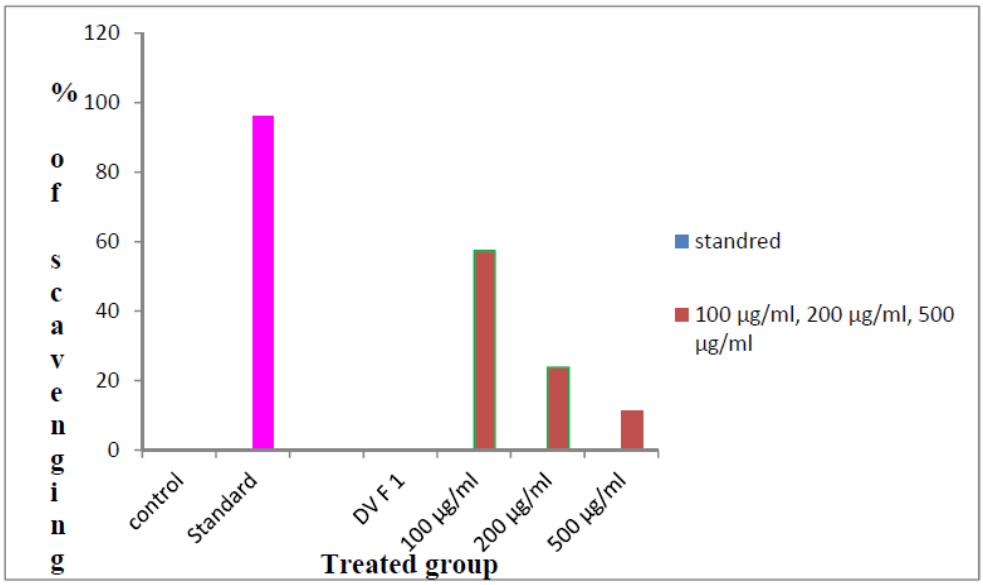


Figure 5. Comparison of Fractions with control and standard groups

Table 3. Antioxidant activities of *Dodonaea viscosa* seeds by SOD method

Gp. No	Treatment	Absorbance at 560nm	Drug % of Inhibition
1	Control	0.6621	0
2	Standard	0.1183	82.13
3	Et-OH	0.4427	33.13
4	CHCL3	0.6777	-2.53
5	Blank	0.0057	99.13

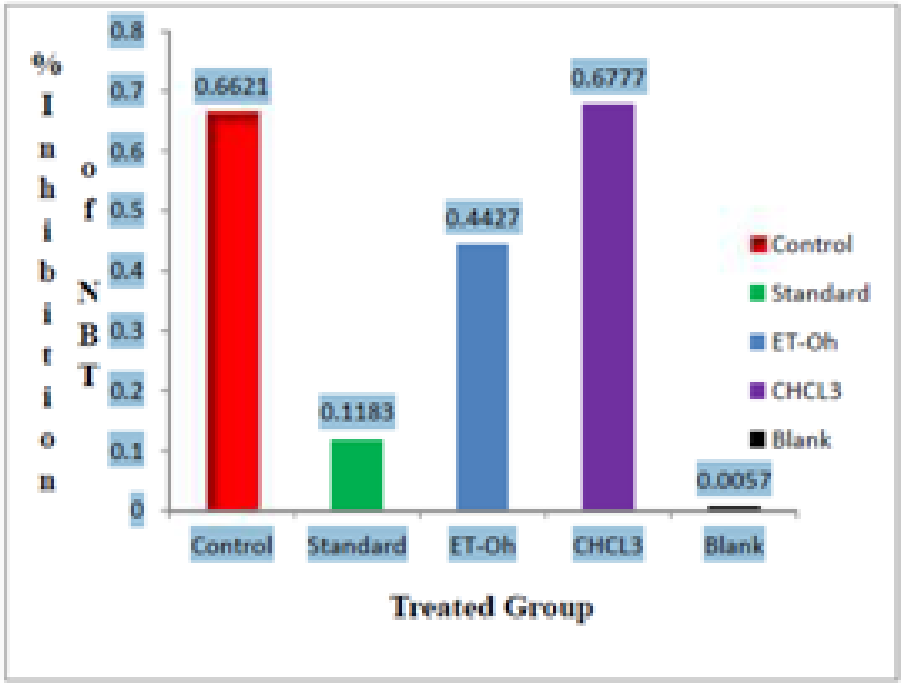


Figure 6. Estimation of Superoxide dismutase in treated groups

Experimental Chemotherapy [16-20]

The tumour inoculation on day 0 (DO), the 0.5 ml of a 1:10 Ehrlich Ascites Tumourcrushed in Hank's balanced salt solution (HBBS) will be inoculated by the i.p., route in albinomice. The treatment began the day after tumour inoculation, and test compounds and vehiclewere administered i.p., using six mice per test group. Routinely, the drug will be administeredsix times on the day after tumour inoculation (D1), on the day D3, D4, D5, D7, D9 and D11(Scheme D1, single dose). In each chemotherapy trail mice were checked daily, with any adverse clinical reactions noted and deaths recorded. Mice are weighed 2-4 times weeklyduring treatment once weekly thereafter. Tumours are measured by Screw gauge twice and tumour volume (mm^3) were estimated as = 0.5 (LengthXWidth2). Results will bepresented for experiments involving eight mice per experimental group.

Evaluation of antitumor activity: Life Span

Mortality will note every day and the median life span will calculated as:

$$MLS = \frac{Dm + (Mm\text{-number of mice dead before Dm})}{\text{Number of mice dead on Dm}}$$

The median mouse (Mm) separates into two identical groups (one group, including the mice that died before Mm, the other group including those who died after) and the median day (Dm) is the day Mm died. Mice surviving for at least 45 days were considered as cured and were included in the calculation of the median life span.

Drug efficiency will express by T/C as follows:

T/C% = (MLS of treated animals/ MLS of animals) × 100 Or

by the increase in life span ILS% = 100 × (T-C) /C

Tumour growth

Treatment efficiency is assessed in terms of the compound′s effects on the tumourvolumes of tumour bearing mice relative to the control vehicle-treated mice. For evaluationcriteria were used in parallel:

(i) Specific tumour growth delay (SGD), calculated as follows:

for Ehrlich Ascites Tumour model = [Td (drug-treated group)]/ Td (vehicle-treated group),Td being the tumour doubling time of drug – treated and control groups, defined as the time in days required for the tumours volume to double.

(ii) Tumour regressions defined as

partial (PR) if the tumour volume decreased to 50% or less of that at the start of the treatment, without dropping below measurable size.

Drugs are administered (p.o) with in different schedules and ILS are then determined.

MTD:Maximum tolerance dose. TI: Therapeutic index, SGD>1 corresponds to minimal level of activity

***IN -VIVO* ANTICANCER ACTIVITY**

For the evaluation of anticancer activity on albino mice are subdivided into ten groups (n=6).

Group-I: Control group- Ehrlichs ascites Carcinoma (EAC) induced by the ip route in albino mice received solvent (p.o.)

Group-II: (EAC) 50mg/kg b.wt of DV-1

Group-III: (EAC) 100mg/kg b.wt of DV-1

Group-IV: (EAC) 200mg/kg b.wt of DV-1

Compounds were first dissolved in DMSO (10 mM) and then diluted in 0.9% NaCl.

Table 8: Anticancer activity of SCFE of given *Dodonaeaviscosa*p.o against the EAC

Treatment schedule	MTD (mg/kg-1)	TI	Dose (mg/kg-1)	SGD	ILS %	Wt(gms) changes in treatment groups		Survival
						0 Day	After 11 day	
D1	>2000	10	50 100	0.66 0.08	14.28 19.04	32.00 30.83	29.16 28.33	3/6 4/6
D 1, 3, 5, 7, 9, 11	>2000	10	50 100 200	0.29 0.20 -0.20	33.33 38.09 52.38	31.00 32.05 32.00	29.50 30.00 29.00	5/6 6/6 6/6

RESULTS AND DISCUSSION

The *Dodonaeaviscosa* of isolated fractions (DV-1) Contain mainly flavonoids. The Rf factor ~4.1 closely resembles specifically they may be flavone or flavanone. The above-mentionedextracts and fractions showedexcellent antioxidant activities at the concentration of 500 µg/ml (81.46%) ,1000 µg/ml (55.27%), Fractions of DVF2 also showed significant inhibition of (84.55%) at 500 µg/ml concentration these results were tabulated in Table 1&2.

The anticancer activity was assessed by determination of percentage of increase lifespan (ILS %) and standard growth delay (SGD) of tumour volume was measured in all the groups. Table 8 summarises the SGD of ethanolic, isolated fractions of *DV*was 0.66,0.08,0.29 and 0.20 respectively. Similarly, the percentage of increase in the life span (ILS %) was 33.33%, 38.09% and 52.35% based on this study*Dodonaeaviscose* of isolated fractions have exhibited prominent anticancer property. Further MTT –assay is also supporting the strong evidence for the activity.

CONCLUSIONS

Dodonaeaviscose of the aerial parts of extracts and isolated fractions contained flavonoids are generally nontoxic and manifest a diverse range of beneficial biological activities like anti-oxidant and antis-neoplastic activities. The tabulated results shown excellent antioxidant and significant decrease in tumour volume and ILS% increase at the of 50, 100 and 200 mg/kg of extracts and isolated fractions. These finding suggest that the presence of flavonoids in *Dodonaeaviscose*aerial parts possess carcinogen in activation, antiproliferation, cell cycle arrest and antioxidation. These promising results were stimulated development of *Dodonaeaviscose* flavonoids for cancer-chemoprevention and chemotherapy.

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