

***Dodonaea viscosa* Jacq: A Medicinal Plant with Cytotoxic Effect on Colon Cancer Cell Line (HT-29)**

Oscar Herrera-Calderon^{1*} , Md. Habibur Rahman² , Gilmar Pena-Rojas³ 
and Vidalina Andia-Ayme⁴ 

¹Department of Pharmacology, Bromatology and Toxicology, Faculty of Pharmacy and Biochemistry, Universidad Nacional Mayor de San Marcos, Lima, Peru. ²Department of Pharmacy, Southeast University, Banani, Dhaka - 1213, Bangladesh. ³Laboratory of Cellular and Molecular Biology, Biological Sciences Faculty, Universidad Nacional de San Cristobal de Huamanga, Portal Independencia 57, Ayacucho - 05003, Peru. ⁴Food Microbiology Laboratory, Biological Sciences Faculty, Universidad Nacional de San Cristobal de Huamanga, Portal Independencia 57, Ayacucho - 05003, Peru.

Abstract

Dodonaea viscosa (Family: Sapindaceae) is a medicinal plant that has been used as anti-rheumatic and bone pain in Peru as a folk remedy. Ethanol extract obtained from *D. viscosa* leaves was partitioned as n-hexane fraction; chloroform fraction; ethyl acetate fraction; n-butanol fraction and aqueous fraction. Phytoconstituents from *D. viscosa* leaves were evaluated by using chemical reagents to identify the presence of each phytochemical. In addition, a cytotoxic effect was determined by the Sulforhodamine B (SRB) test. The results showed that the ethanol extract and ethyl acetate fraction of leaves had the highest variety of phytoconstituents. The ethanol extract of leaves exhibited a major inhibitory effect compared to different fractions in human colon cancer cells (HT-29). From these assays, it is concluded that *D. viscosa* does not possess any detectable cytotoxic effect on epidermal cells from mouse (3T3), and a slight cytotoxic effect against HT-29 tumor cells compared to 5-FU. This species could appear like a good source of herbal medicine in colorectal cancer disease.

Keywords: *Dodonaea viscosa*, colon cancer, flavonoids, phytochemicals, cytotoxic activity

*Correspondence: oherrera@unmsm.edu.pe; +51 95 6550510

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INTRODUCTION

Colon cancer or colorectal cancer (CRC) ranks as third cancer in incidence and the second in mortality worldwide¹. CRC is the result of uncontrolled cell growth in a specific part of the large intestine produced by multiple factors, which are involved in the development of colorectal cancer such as excessive alcohol consumption, family history, lack of physical activity, high-fat diets with no fiber old age, red meat, diabetes, and inflammatory bowel diseases (IBD), including ulcerative colitis and other chronic diseases linked to large intestine². In 2018, the Global Cancer Observatory (GLOBOCAN) estimates 18.1 million new cancer cases as well as 9.6 million cancer deaths³.

The relationship between human beings and nature is very known. Human beings get medicine from nature because they are continuously digging the knowledge from different plants and their effectiveness as treatment of several non-transmissible diseases⁴. Most of the phytochemicals are bioactive and can be used in the production of synthetic medicine or druggable options in the antitumor drug discovery.

Screening of drug in colon cancer studies have been carried out on HT-29, HCT116, and Caco-2 cells. HT29 is known as human colon adenocarcinoma cell line and shows a biochemistry of mature intestinal cells. Additionally the medicinal plants used as anti-inflammatory coming from the ethnopharmacological knowledge and they are tested on this type of tumor cells, taking into account its toxicity and effective concentration⁵. On the other hand, the factors

secreted by HT29 cells are growth factors, platelet-derived growth factor, chemokines, immunomodulatory and pro-inflammatory cytokines⁶. Studies of natural products as flavonoids⁷ and terpenes⁸ have demonstrated regulation of these components linked to the inflammation process.

The discovery of new drugs for tumor treatment is focused on its tolerability and safety profile, some of them were found in natural sources⁹. The preclinical phase of antitumor drugs generally are tested *in-silico* and *in-vitro*, and its properties can vary such as anti-mutagenic, anti-proliferative, cytotoxic, and pro-apoptosis. Therefore, validation of any it must be alternative therapy based on medicinal plants has gained much interest due to its availability, accessibility, affordability and have less side effects¹⁰. On the other hand, a clinical trial is the last step to ensure its use in the population. Several medicinal plants of diverse families have therapeutic properties, which improve and decrease high rates of morbidity and mortality.

Dodonaea viscosa Jacq. (Family: Sapindaceae) is a shrub, rarely a small tree, and widely distributed in tropical and subtropical areas of both hemispheres¹¹. It is used in folk medicine as a febrifuge, a diaphoretic drug, and for the treatment of rheumatism, gout, inflammations, swelling, and pain in Asian countries. According to Peruvian folk medicines, *D. viscosa* leaves (Fig. 1) are used as an anti-inflammatory and analgesic herbal medicine. In the Apurimac region, *D. viscosa* is known as "chamana" and grows at 2300 meter above the sea level (m.a.s.l). Several flavonoids, diterpenoid acids and saponins have been found



Fig. 1. Aerial parts of *Dodonaea viscosa* Jacq.

in this species such as 3,3',4',5,7-pentahydroxy flavane, hautriwaic acid, 4-methoxystigmasterol, 5-hydroxy-7,3',4'-trimethoxy-6-acetoxy-3-prenylflavone, dodonic acid, dodoviscins¹² and other phytochemicals determined in the literature (Fig. 2a and Fig. 2b). Nowadays, the cytotoxic effect of *Dodonaea viscosa* *in vitro* has not been tested for colon cancer. We proposed to validate the medicinal potential of this plant, evaluating the different fractions obtained from *Dodonaea viscosa* leaves on HT-29 tumor cells.

MATERIALS AND METHODS

Chemicals

Dulbecco's Modified Eagle Medium (DMEM), Sulforhodamine B (SRB), and fetal calf serum were purchased from Sigma-Aldrich, USA. Other required chemicals of ACS grade were obtained from the local market of chemical companies.

Plant material

Aerial parts of *Dodonaea viscosa* were collected from Chalhuanca, Aymaraes, Apurimac

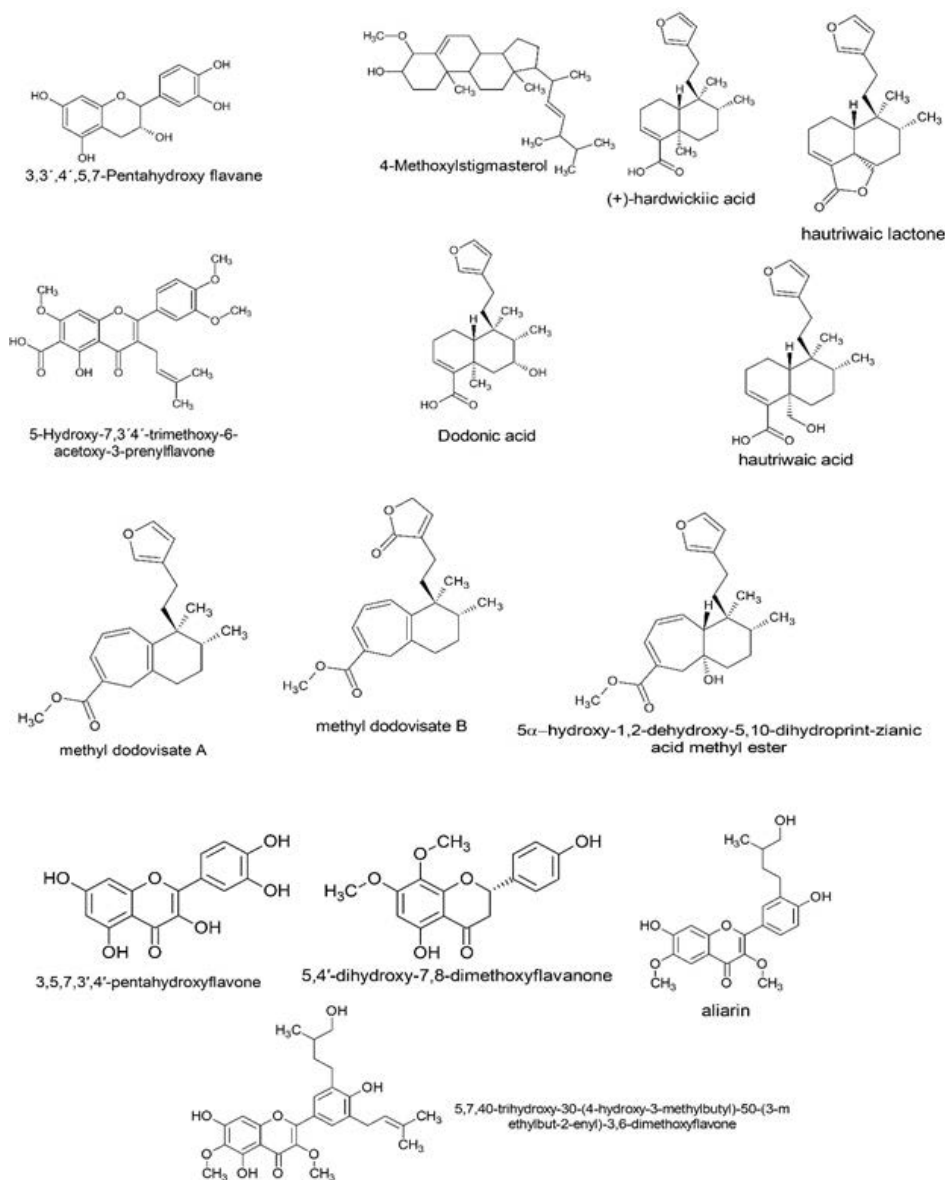


Fig. 2a. Chemical constituents reported in *Dodonaea viscosa* Jacq leaves

Table 1. Qualitative phytochemical screening for *Dodonaea viscosa Jacq*

Constituents	Tests	Result	Results					
			n-hexane	Chloroform	Ethyl acetate	n-butanol	ethanol	Aqueous
1. Sterols	Salkowski Test	Chloroform Layer: Red Acid Layer: Greenish yellow fluorescence	+	+	+	+	+	-
2. Terpenoids	Lieberman-Burchardat Test	Reddish brown color at the interface	+	+	+	+	+	-
3. Alkaloids	Mayer's Test	Creamy-white precipitates	-	+	+	+	+	+
	Wagner's Test	Reddish-brown precipitates	-	+	+	+	+	+
4. Carbohydrates	Molisch's Test	Purple to violet color ring at junction	+	+	+	+	+	+
5. Flavonoids	Fehling's Test	Brick red color precipitates	+	+	+	+	+	+
	Shinoda Test	Red color	-	+	+	+	+	+
	Aluminum Chloride Test	Yellow color, becomes colorless when NaOH and HCl is added.	-	+	+	+	+	+
	Gelatine Test	White precipitates	-	-	-	+	+	+
6. Tannins	Lead Sub Acetate Test	Creamy gelatinous precipitates	-	-	-	+	+	+
7. Phenols	Ferric Chloride Test	Blue color	-	+	+	+	+	+
8. Saponin	Foam Test	Foam layer	-	-	-	+	+	+
9. Steroids	Chloroform test	Red color in chloroform layer	+	+	+	+	+	-

(+) positive; (-) negative.

region, Peru during the month of July 2019 and it was successively identified by Mg. Asuncion A. Cano Echevarria, a voucher specimen (Id 125-USM-2019) was deposited at the Museum of the Natural History of the Universidad Nacional Mayor de San Marcos (UNMSM, Lima, Peru).

Extraction and fractionation of plant materials

The aerial parts (~500 g) were dried, powdered, and macerated for 7 days with 96% ethanol at room temperature and then filtered with Whatman filter paper Grade 1. The filtrate was concentrated by removing the solvent by using rotary evaporator under vacuum at 40°C and 80 RPM, resulting 50.2 grams of dark green ethanolic extract. Ethanolic extract was prepared with 600 mL distilled water, generating a suspension and it was separated into various fractions by using solvents of increasing polarity in separating funnel, resulting in *n*-hexane (4g), Chloroform (5g), Ethyl acetate (11 g), *n*-butanol (10g) and aqueous (20g) fractions.

Preliminary Phytochemical analysis

Preliminary phytochemical analysis of the ethanolic extract and fractions were carried out for the presence of alkaloids, carbohydrates, flavonoids, glycosides, sterols, terpenoids, tannins, phenols and saponin by using standard procedures¹³.

Cytotoxicity effect

Cell culture

HT-29 tumor cell lines from human colon adenocarcinoma and 3T3 from non-tumorigenic, BALB/c mouse embryo cells, cell lines were cultivated at the Laboratory of Immunology and Virology, Universidad Peruana Cayetano Heredia (UPCH). HT29 and 3T3 cell lines were grown in Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum and as an antibiotic was used 50 µg/mL gentamycin in humidified 5% CO₂/95% air at 37°C under good laboratory practices conditions.

Cytotoxicity assay

The cytotoxic activity was performed by using the procedure of de Carvalho *et al.*¹⁴ A 96-well tissue culture plate was taken and each of its well was inoculated with 3000-5000 cells in their corresponding medium and was incubated at 37°C for 24 hours. Meanwhile, a mixture of the crude ethanolic extract and fractions (hexane fraction; chloroform fraction; ethyl acetate fraction; butanol

Table 2. Cytotoxicity of ethanolic extract and fractions of *Dodonaea viscosa* on human tumor cell lines

Cytotoxic samples	Values of IC ₅₀ (µg/mL) ^A	
	Tumor cell lines HT-29	Mouse embryo normal cells 3T3
1	10.52 ± 2.5*	38.21 ± 2.1
2	>250	40.11 ± 1.1
3	15.12 ± 2.0*	182.14 ± 2.1
4	38.52 ± 2.69	25.0 ± 2.0
5	40.22 ± 2.0	35.4 ± 1.0
6	120.00 ± 5.0	100.8 ± 1.5
5-FU	0.33 ± 0.01	< 0.24

1= ethanolic extract; 2= *n*-hexane fraction; 3= chloroform fraction; 4= ethyl acetate fraction; 5= *n*-butanol fraction; 6= aqueous fraction. 5-FU=5-Fluorouracil.

^AValues were expressed as mean ± SD (n = 3). *P < 0.05, significant difference as compared to standard drug 5-FU.

fraction and aqueous fraction) with concentrations equivalents to 0-250 µg/mL and as control drug 5-FU (0-62.5 µg/mL) were dissolved in dimethyl sulfoxide and incubated at 37°C with 5% CO₂ and 95% air for 48 hours. HT-29 and 3T3 cell lines were fixed with 10% trichloroacetic acid (TCA) and was stained with sulforhodamine B (SRB) dye for 20 minutes. Cells were washed with 1% acetic acid to remove the excess dye and subsequently the protein-bound dye was solubilized by adding 10 mM solution of Tris buffer (pH 10.5). Finally, samples were read at 510 nm by using a microplate reader. The results obtained by triplicate were expressed as inhibitory concentration 50 (IC₅₀), which was determined by linear regression analysis.

Statistical analysis

The cytotoxic effect against two cell lines (HT-29 and 3T3) were analyzed three times and the results were expressed as mean ± standard deviation (SD). For graphical and statistical analysis, SPSS 22.0 and Microsoft Excel 2016 were used as statistical software. One-way ANOVA followed by a post hoc Tukey test were used as statistical test with a significance P < 0.05.

RESULTS

Determination of Phytochemical constituents

Qualitative screening of *D. viscosa* for

the presence of phytochemical constituents was carried out by using its crude ethanolic extract and fractions. The results are presented in Table 1, which shows that the plant has alkaloids, sterols, carbohydrates, terpenoids, flavonoid, tannins, phenols, glycosides and saponin.

Cytotoxic assay

The IC₅₀ values of the ethanolic extract and fractions of *D. viscosa* leaves as well as 5-FU (antitumor drug) on tumor cell lines are shown

in table 2. The chloroform fraction and ethanol extract showed IC₅₀ values below of 20 µg/mL for HT-29 cell lines, but less than 5-FU. All fractions and crude ethanol extract did not present any evidence of cytotoxicity on 3T3 cell line.

DISCUSSION

Several phytochemical studies of *D. viscosa* have been reported, which determined the presence of flavonoids, terpenes, lactones, and

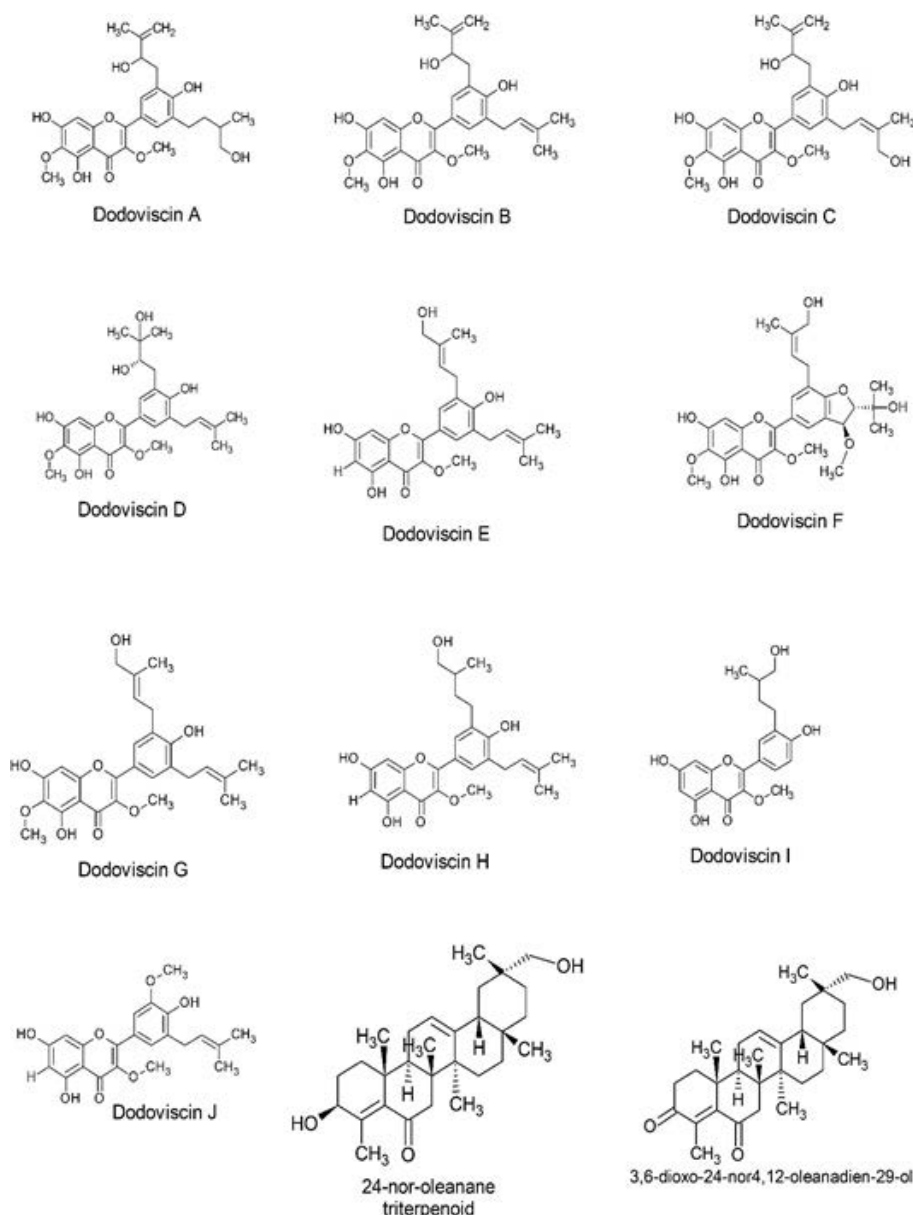


Fig. 2b. Chemical constituents reported in *Dodonaea viscosa* Jacq leaves

alkaloids. Our results based on fractions are similar to other works reported in the literature. From the fraction chloroform extracted one flavonoid named 3, 5, 7, 3',4'-pentahydroxy-flavone, hautriwaic acid. These compounds showed a high antimicrobial activity against gram positive and negative bacterial¹⁵.

Prenylated flavonoids present in *D. viscosa* leaves have demonstrated anti-inflammatory effect due to several types of Dodoviscins isolated of the soluble fraction from the ethyl acetate extraction¹⁶. Otherwise, exist a relationship between the gut microbiota and colorectal cancer, when this microbiota is altered a chronic inflammation could be the starting point of the colorectal cancer progression^{17,18}.

The cytotoxic effect showed for ethanolic extract and chloroform fraction could be linked to phytochemical constituents such as phenolic compounds, flavonoids, tannins, saponins or alkaloids; furthermore, many reports attributed that flavonoids consumption could decrease the risk of colorectal cancer¹⁹, while it is very difficult to argue that the same can occur with other types of cancer as breast cancer or lung cancer. Otherwise, quercetin, myricetin, apigenin and luteolin was not associated between their consumption and the incidence of any type of cancer or mortality from this cause. Although, a positive relationship was found between their intake and mortality from stomach cancer²⁰. The results showed in Table 2 corresponds to values less than 20 µg/mL and according to the U.S. National Cancer Institute (NCI), both extracts are recognized as potential cytotoxic agents, although is necessary to evaluate in another colorectal cancer cell line²¹.

There were several studies related to cytotoxicity of *D. viscosa*, an in-vitro study showed that the synthesized AuNPs (gold nanoparticles) using *Dodonaea viscosa* leaf extract inhibited the growth of A549 NSCLC cells (adenocarcinomic human alveolar basal epithelial cells), being the most active the methanol extract with the IC₅₀ values of 4.0 µg/ml²². Dodoneasides A and B isolated from the ethanol extract of *D. viscosa* roots showed antiproliferative activity against the A2780 human ovarian cancer cell line²³. Finally, Dodoviscin A isolated of the aerial parts from *D. viscosa* inhibits melanogenesis in mouse b16-f10 melanoma cells²⁴.

Our limitations in this study are focused on the cytotoxic activity due to only one tumor cell line of colorectal cancer was used in the assay and we did not isolate the phytochemicals of the ethanol extract and its fractions.

CONCLUSION

This study concluded that the ethanolic extract and the chloroform fraction from *D. viscosa* presented a cytotoxic effect on HT29-cell line with values less than 20 µg/mL. Furthermore, our study did not show cytotoxicity on 3T3 cells, which it could demonstrate that the phytochemicals of *D. viscosa* are selective to colorectal cancer cells. Further studies are needed to complement mechanisms and experimental studies with rodents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All listed author(s) have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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None.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

DATA AVAILABILITY

Not applicable.

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