

# Chromatographic Fingerprinting, Antioxidant, and Anti-inflammatory Potential of *Dioscorea villosa* (Wild Yam) Leaves

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## Abstract

**Context:** Free radicals have been implicated in a wide range diversity of diseases and ailments, and therefore, the compounds having the ability to scavenge these free radicals are under extensive investigation, of which *Dioscorea* species have been actively involved. **Objective:** The current study assessed the anti-inflammatory and antioxidant potential of standardized leaf extracts of *Dioscorea villosa*. **Material and Methods:** Anti-inflammatory activity was carried out using carrageenan-induced paw edema assay, and antioxidant activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay. Chromatographic fingerprinting of the crude methanolic extract was carried out using high-performance liquid chromatography (HPLC) whereby diosgenin was used as a standard marker compound. **Results:** Among all the crude successive extracts, methanolic extract showed significant anti-inflammatory activity in comparison with the standard whereby the extract showed maximum inhibition of paw edema after 3 h of carrageenan injection. The aqueous extract showed noticeable antioxidant activity with the half maximal inhibitory concentration of 21.36 µg/ml. **Discussion:** The preliminary phytochemical screening showed the presence of flavonoids and tannins that may be responsible for the observed effect. In addition, the presence of steroids marks toward the observed anti-inflammatory activity. In addition, the extract showed noticeable levels of diosgenin, which were marked and quantified using HPLC. **Conclusion:** The results strongly support the ethnobotanical use of the plant.

**Key words:** Chromatographic fingerprinting, *Dioscorea villosa*, dioscoreaceae, diosgenin

## INTRODUCTION

The genus *Dioscorea* (Dioscoreaceae), also called as yam, involves around 600 species scattered throughout the world.<sup>[1]</sup> A large number of species contain steroidal saponins and furthermore sapogenins, for example, diosgenin, which constitutes in industrial synthesis as the crude material for the production of many steroids that are presently accessible in the worldwide medicinal markets as anti-inflammatory, androgenic, estrogenic, and contraceptive drugs. Furthermore, these classes of compounds are also reported to possess cytotoxic, antitumor, antifungal, immunoregulatory, hypoglycemic, and cardiovascular properties.<sup>[2-5]</sup>

*Dioscorea villosa* (wild yam) has cyclinder tubers with twining vine which is pale-brown, knotty, and woody in nature. The tubers are twisted and bore horizontal branches. It is having a reddish stem which is thin and grows to a length of over 9.2 m. This plant produces bunches of greenish-white or greenish-yellow flowers. The leaves of this plant are heart-shaped, symmetrical and having smooth top surface,

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gradually tapering to a sharp, and acuminate point. The upper part of the leaves is irregular alternate while the lower leaves are present in whorls of 4 or 5. The rhizome of *D. villosa* appears to be slender and 5 contorted pieces which are of an inch to 1/2 an inch in diameter and often 2 feet in length. It is structurally oval, and because of being flattened, it creeps horizontally under the earth surface. It is an evergreen plant which is a low creeper and occupies from average to poor soil.<sup>[6]</sup>

Wild yam is accepted to be useful to the liver and endocrine framework. It controls the female framework, especially amid menstrual trouble and menopause, and utilized as a part of treating infertility. Activities such as antispasmodic and anti-inflammatory of rhizomes make it valuable in treating symptoms of stiffness and joint inflammation, and these characteristics make it helpful in treating abdominal cramps and muscle pain. It has additionally been utilized in the treatment of stomach-related disorders including gallbladder irritation, irritable bowel syndrome, and diverticulitis.<sup>[7]</sup> The mixture of its diuretic and anti-inflammatory actions makes it a good choice for urinary tract conditions. Wild yam contains diosgenin which is utilized as a part of birth pills and other steroidal hormones. This study is based on the theory that wild yams might help to regulate the female sex hormones, and therefore, it is considered a great herb to treat the symptoms commonly associated with menopause. It is used as an antispasmodic and therefore finds application in abdominal cramps, cough, and hiccups and for muscular spasms, croup, and flatulence. It is useful in the treatment of loosening phlegm, inducing emesis and increasing urine output.<sup>[8]</sup>

The vitality of this drug appears to reside in an acrid resin which is almost insoluble in water but readily soluble in alcohol. Dioscin is simply a dried solid extract and not the active principle present in rhizome. Wild yam contains a huge quantity of starch. *Diosgenin* is the primary active present in *Dioscorea* and is structurally similar to cholesterol. On oral administration, initially, it is metabolized in the liver and later eliminated through bile. Due to the structural similarity of diosgenin with estrogenic precursors, it is believed to have estrogenic and anti-inflammatory effects. Dioscin is the glycosidic form of diosgenin, and it may have closely related effects to diosgenin. The major tuber storage protein of *Dioscorea* is Dioscorin,<sup>[9]</sup> but no known physiological effects have been found till date.

## MATERIALS AND METHODS

### Plant material

*D. villosa* leaves were collected from herbal garden of Lovely Professional University and authenticated by H.B. Singh, taxonomist at National Institute of Science

Communication and Information Resources (NISCAIR), New Delhi, and a voucher specimen (no. NISCAIR/RHMD/Consult/-2010-11/1568/166) has been retained at the Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab (India).

### Preparation of extracts

The coarsely powdered dried plant material (20 g) was successively extracted on small scale with petroleum ether (300 ml) at 60°C, chloroform (300 ml) at 63°C, methanol at 64°C, and water at 100°C for 5 h using Soxhlet apparatus. The extracts after filtration were evaporated to dryness under reduced pressure and made to a specified volume.<sup>[10]</sup>

### Phytochemical investigation

The leaves of the plant were subjected to phytochemical investigation based on the method described by Ahmad and Beg, 2001 for the detection of alkaloids, flavonoids, steroids, glycosides, tannins, and saponins.<sup>[11]</sup>

### Chromatographic fingerprinting

Methanolic extract was subjected for chromatographic fingerprinting using high performance liquid chromatography (HPLC). Stock solution of the extract of 0.1 mg/ml was prepared using acetonitrile and further diluted to obtain a concentration of 100 µg/ml. The solution was sonicated for 15 min. About 1 ml of the sample solution was filtered through a 0.45 µm filter membrane before analysis. Stock solution of standard marker compound from *D. villosa*, namely, diosgenin was prepared in the same manner and was further diluted serially to obtain a concentration of 100 µg/ml.

HPLC (Shimadzu, Japan) equipped with a photodiode array detector was used for fingerprinting. Data acquisition and processing were performed by LC solutions 10V software. Chromatographic separation was carried out at room temperature using a Phenomenex C18 (300 mm × 3.9 mm) analytical column. The mobile phase consisted of water:acetonitrile in a gradient elution of (70:30, 55:45, 30:70, and 70:30) within a run time of 40 min.

### Antioxidant activity

Methanol and aqueous extracts were used to evaluate the antioxidant activity. Ascorbic acid at equal concentration was used as standard drug. Methanolic solution of the extracts as well as ascorbic acid (standard) at the concentration of 10, 20, 40, 60, 80, and 100 µg/ml was used for the evaluation of antioxidant activity. The scavenging effect of *Dioscorea* species and ascorbic acid corresponding to the quenching intensity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was

carried out. Aliquots of 1 ml wild yam leaves extract and 5 ml of freshly prepared 0.1 mM DPPH methanolic solutions were thoroughly mixed and kept for 50 min in the dark. The absorbance of the reaction mixture at 520 nm was noted with a spectrophotometer. Methanol (1 ml), replacing the extract, was used as the blank. The percentage of free radical scavenging effect was calculated as follows:

Scavenging effect (%) =

$$\frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

Control - DPPH in methanol

Sample - methanolic extract and DPPH (Ma *et al.*, 2005).

### Anti-inflammatory activity

Aqueous and methanolic extracts of the plant were subjected to evaluate the anti-inflammatory activity.

The extracts were dissolved in distilled water and 2.5% Tween 80 in water, respectively, just before use.

### Animals

Wistar albino rats of either sex weighing 180–300 g bred in the National Institute of Pharmaceutical Education and Research, Mohali, were used. In clean polyacrylic cages of animal house, the specimens were housed in a group of five. The animals were housed under 12 h light and dark cycle with an average temperature of  $25 \pm 2^\circ\text{C}$  and humidity of 55–65%. The rats were fed with commercial pelleted animal feed supplied by M/s Hindustan Lever Ltd., Bengaluru, India, and free access of water.

### Carrageenan-induced rat paw edema

Rats are divided into four different groups and injected in the right paw of each specimen under the sub-plantar region with carrageenan (0.1 ml, 1%) to check the influence of the extract on acute inflammation. After that, they were dispensed the extract (200 mg/kg), orally 1 h before carrageenan injection. An equal volume of distilled water (10 ml/kg) and Tween 80 (2.5%) was used as control and acetylsalicylic acid (150 mg/kg) was used as reference. Edema is measured by increased in linear paw circumference.<sup>[12]</sup>

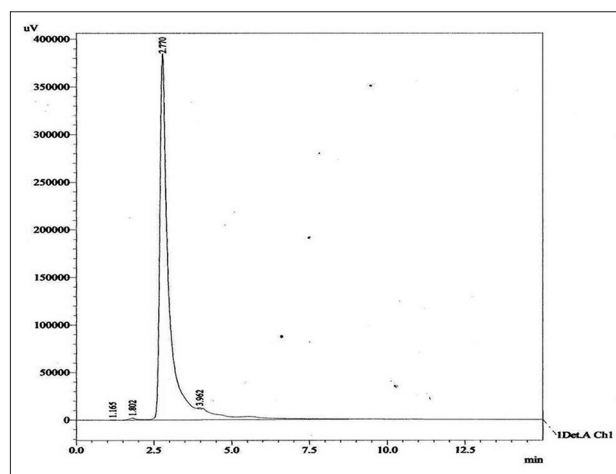
### Statistical analysis

Each group consisted of five animals. The data were expressed as mean  $\pm$  standard error of the mean. All the extracts were compared with control group using one-way analysis of variance followed by Dunnett test.  $*P < 0.05$  was considered statistically significant.

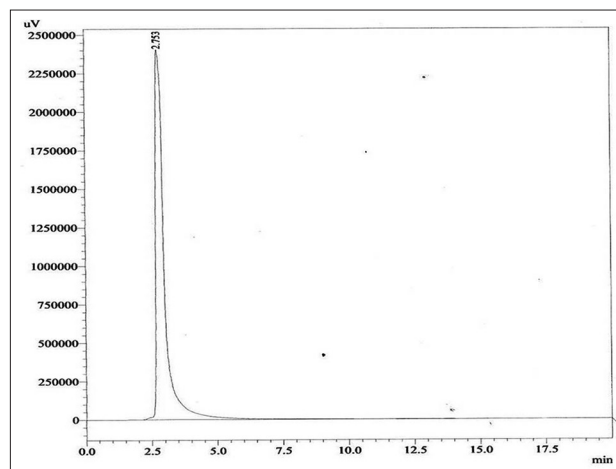
## RESULTS

Alkaloids, glycosides, flavonoids, tannins, and saponins were found to be positive in methanolic and aqueous extract, whereas steroids were found only in the methanolic extract as a result of phytochemical investigation of *D. villosa* leaves. Hence, it can be generalized that the major constituent in *D. villosa* leaf extract (methanolic) is diosgenin. Therefore, HPLC fingerprint analysis was performed based on the relative retention time. The retention time of major peak in crude extract is 2.77 and that of diosgenin was 2.75. Standardization of 100  $\mu\text{g}$  of crude methanolic extract showed the presence of 15.2% diosgenin. A representative HPLC fingerprint of crude extract and standard marker, namely, diosgenin are shown in Figures 1 and 2, respectively. The HPLC detection of active constituent based on the chromatographic retention time confirms the presence of active moiety, diosgenin, that is responsible for the claimed pharmacological activities of the drug.

The DPPH radical scavenging effects of yam leaf extracts are presented in Figure 3. Both extracts showed evident



**Figure 1:** Chromatogram of crude methanolic extract showing peak of *diosgenin*

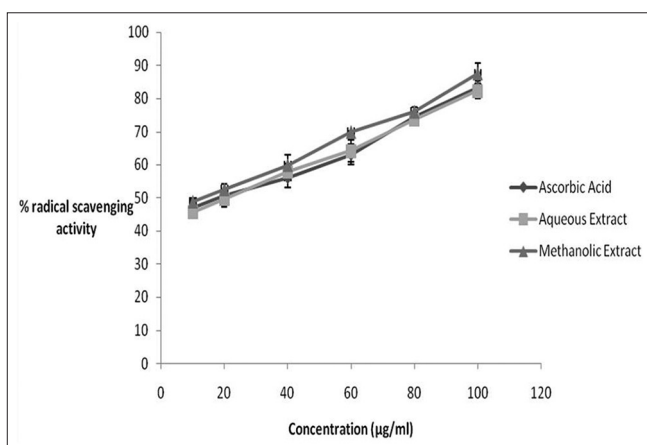


**Figure 2:** Chromatogram of standard marker compound, namely, *diosgenin*

**Table 1:** Comparative anti-inflammatory activity of extracts

Treatment	Paw size (cm.)				
	0 h	1 h	2 h	3 h	4 h
Control	0.58±0.037	0.66±0.050	0.64±0.040	0.56±0.024	0.54±0.024
Standard	0.56±0.040**	0.58±0.020**	0.48±0.020**	0.44±0.024**	0.44±0.024**
Aqueous	0.54±0.024**	0.56±0.050**	0.50±0.044**	0.44±0.024**	0.40±0.020**
Methanol	0.50±0.031**	0.50±0.031**	0.42±0.020**	0.40±0.031**	0.38±0.037**

Values indicated represent paw size (cm). Values are mean±SEM  $n=5$ , Significant at \* $P<0.05$ , \*\* $P<0.01$  (one-way ANOVA, Dunnett: Compare all vs. control applied using INSTAT software). ANOVA: Analysis of variance, SEM: Standard error of the mean

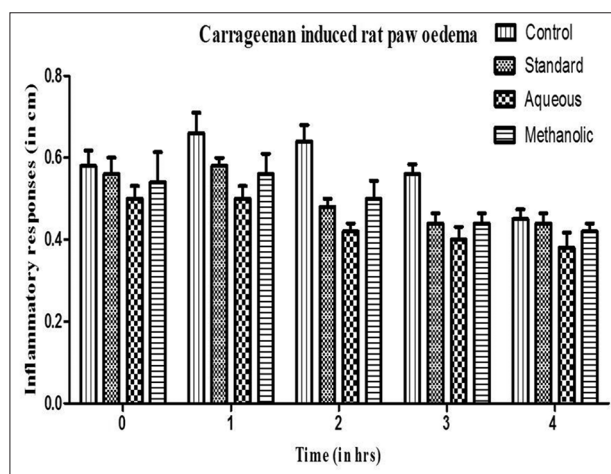

**Figure 3:** Comparative antioxidant activity of crude extracts

free radical scavenging activities. In DPPH free radical scavenging assay, the aqueous extract showed the noticeable radical scavenging 82.37% at the concentration of 100 µg/ml. A linear dose-response relationship was observed whereby the activity increased with increase in the concentration of each individual yam extract. The half maximal inhibitory concentration of the ascorbic acid, aqueous, and methanolic extracts was calculated to be 20.32 µg/ml, 21.36 µg/ml, and 40.24 µg/ml, respectively.

All the extracts showed significant inhibition of paw edema induced by carrageenan, a polysaccharide, that acts through various mechanisms to induce inflammation. However, the induced inflammation is short lived, and it has been suggested that the drug action is quickly metabolized and eliminated from the system after reaching its peak in 1 h. Both leaf extracts showed significant inhibition of paw edema (\* $P<0.05$ ) at the tested concentration of 200 mg/kg [Table 1] as determined by the paw volume.

## DISCUSSION

The presence of alkaloids, flavonoids, glycosides, saponins, phytosterols, tannins, and steroids in *D. villosa* was confirmed after phytochemical screening. The combined effect of flavonols and tannins may be responsible for scavenging the free radicals as observed with the methanolic extract. The samples were evaluated by DPPH radical scavenging


**Figure 4:** Comparative anti-inflammatory activity of crude extracts

test. The increased presence of free radicals is the cause of almost all human diseases such as cardiovascular diseases and cancer. Therefore, the dietary antioxidants present in wild yams are active in fighting against the damage to cellular DNA, lipids, and proteins caused by free radicals. The new trends of consuming food with specific health benefits support the idea of consumption of plants containing antioxidants to treat diseases such as coronary heart diseases and cancer.<sup>[13]</sup> Apart from the multiple biological effects, the phenolic compounds also have antioxidant property. The diversified classes of antioxidants in fruits and vegetables make it difficult to measure each antioxidant component separately. Hence, various methods have been developed over the past few years to determine the antioxidant activity of biological samples.<sup>[14]</sup> From this study, both the extracts were found to be active with regard to free radical scavenging activity. Both extracts exhibited better antioxidant activity than the standard.

The two extracts of *D. villosa* were evaluated for anti-inflammatory activity using carrageenan-induced paw edema in the increasing order of methanol>aqueous>standard as shown in Figure 4. The anti-inflammatory activity can be attributed to the steroidal content of the extracts. Moreover, interestingly, the extract showed more prominent effect as anti-inflammatory drug in comparison to the standard aspirin.



## CONCLUSION

The crude extracts of *D. villosa* leaves showed significant antioxidant and anti-inflammatory activities. Upcoming studies aim to isolate and classify the components responsible for the observed effects. However, the results observed strongly support the ethnobotanical use of the plant.

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