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EVALUATION OF IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF THE LEAVES OF *DIOSCOREA HISPIDA*

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ABSTRACT

Dioscorea hispida, also known as the Indian three-leaved yam, is a species of yam in the genus Dioscorea, native to South and Southeast Asia. The present study highlights the anti-inflammatory activity of the *Dioscorea hispida* leaves. The phytochemical screening of the aqueous and alcoholic extracts of the leaves were performed and revealed the presence of Alkaloids, phenolic compounds and saponins. The invitro anti-inflammatory activity of total aqueous and total alcoholic extracts were performed by protein denaturation method using diclofenac as standard. The total alcoholic and total aqueous extracts shown to have significant anti- inflammatory activity.

Key Words: *Dioscorea hispida*, anti- inflammatory, protein denaturation.

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INTRODUCTION

Medicinal plants are the integral component of the pharmaceutical industry. India has ancient heritage of traditional medicines. The herbal drug constitute a major part in all traditional system of medicine. Indian material medical include 2000 natural product of the therapeutic importance of which 400 are mineral and animal origin. The extraction is the separation of medicinally active protein of plant tissue using selective solvent through standard procedure. The product is obtained from plant are relatively complex mixture of metabolites in liquid or semi-solid or in dry powder form and are indented for oral or external use (1, 2). *Dioscorea* species popularly known as yam. In India it is a prime staple medicinal food substitute for the majority of rural and local people. The tubers of *Dioscorea hispida* used to kill worms in wounds. The present study mainly focusing on the anti-inflammatory action on the *Dioscorea hispida* leaves. The inflammatory response represents a generalized response to infection or tissue damage and designed to remove cellular debris to localize invading organism and arrest the spread of infection. The inflammatory response is characterized by the following symptoms: Reddening of the localized area, swelling, pain and elevated temperature (3).

MATERIALS AND METHODS

Plant collection and drying

The plant *Dioscorea hispida* were collected from Malappuram. The plant material was taxonomically identified by the botanist, Dr. Anil Kumar V S. HOD, Department of botany, Government College, Kasaragod. The plant dried under for shade for about 7 days and then powdered with mechanical grinder and stored in an air tight container.

Preliminary phytochemical screening (4, 5)

Extraction of the dried powder of the *Dioscorea hispida* leaves was carried out by soxhlet extraction method using alcohol and water. Each extract was then filtered, the solvent distilled off and finally the dried extract was obtained. This extract were used for preliminary phytochemical screening reveals the presence of alkaloids, phenolic compounds and saponins. **Extraction of aqueous and alcoholic leaf extract of plant (4, 5)**

The total alcoholic extract was prepared by using Soxhlet extraction method. Total aqueous extract was prepared by maceration method.

Anti-inflammatory activity (6, 7, 8)

Inhibition of protein denaturation method

The reaction mixture (0.5ml) consist of 0.45ml bovine serum albumin (5% aqueous solution) and 0.05ml of plant extract 50,100,150,200 µg/ml concentration (total aqueous and alcohol) and pH was adjusted to 6.3 using 1N HCl. The sample were incubated at 37°C for 20min and then heated at 57°C for 3min. Diclofenac was used as standard drug (50,100,150,200µg/ml). After cooling the samples, 2.5ml phosphate buffer saline (pH 6.3) was added to each tube. Absorbance was measured spectrophotometric ally at 660 nm. For control 0.05ml distilled water was used instead of extract while product controlled leaked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

Percentage inhibition = [(A○ control_ A1 sample/standard) / A○ control) x 100

Where, A○ = absorbance of the control

A1 = absorbance of the sample/ standard

Calculation of IC50 (50% inhibitory concentration)

The concentration (µg/ml) of the drug required to denature 50% protein was calculated from the graph. The IC50 value was calculated for inhibitory concentration of the sample and standard.

RESULTS AND DISCUSSION

Protein denaturation method

In this method, the total aqueous and alcoholic extract of *Dioscorea hispida* leaves (50, 100, 150 and 200 µg/ml) displayed significant activity. The extract at concentration of 200 µg/ml shows maximum activity, the result is shown in table-1 and Fig-1.

Table-1 Result of *in vitro* anti-inflammatory activity of total aqueous and total alcoholic leaf extracts of *Dioscorea hispida* by protein denaturation method.

SI. no	Sample	Concentration (µg/ml)	Absorbance at 660 nm	% inhibition
1	Control	-	0.267	-
2	Standard (Diclofenac)	50	0.138	48.30
		100	0.110	58.80
		150	0.095	64.41
		200	0.080	70.03
3	Total alcoholic Extract	50	0.178	26.70
		100	0.140	47.56
		150	0.130	51.31
		200	0.085	68.16
4	Total aqueous Extract	50	0.147	44.94
		100	0.112	58.05
		150	0.093	65.16
		200	0.091	65.91

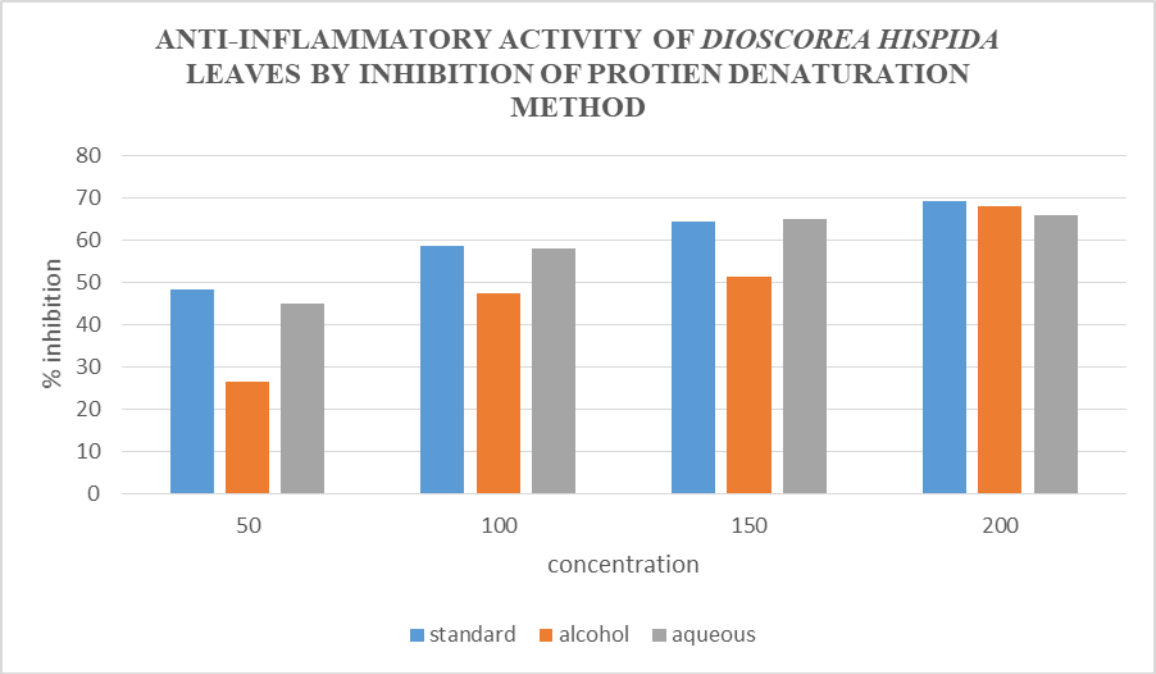

Fig-1 results showing *in vitro* anti- inflammatory activity of total aqueous and total alcoholic leaf extract of *Dioscora hispida* by protein denaturation method

IC 50 value was calculated for aqueous and alcoholic extract of *Dioscorea hispida* and standard from the graph. The value are tabulated in table-2

Table-2 Result of *in vitro* anti-inflammatory activity of total aqueous and total alcoholic leaf extracts of *Dioscorea hispida* by protein denaturation method

	Sample	IC50 (mcg /ml)
1	Standard	52
2	Alcoholic extract	148
3	Aqueous extract	75

CONCLUSION

The air dried powder drug subjected to Soxhlet extraction using methanol and distilled water as per standard procedure. The total alcoholic and aqueous extract obtained from Soxhlet extraction were used for preliminary phytochemical analysis it reveals the presence of alkaloids, phenolic compound and saponins. The *in-vitro* anti-inflammatory activity also performed by protein denaturation method. The total aqueous extract of plant showed more significant activity, when compared with standard diclofenac sodium than that of total alcoholic extract. The extracts at the concentration of 200µg/ml shows maximum activity.

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