



## Research Article

### **IN VITRO EVALUATION FOR IMMUNOMODULATORY ACTIVITY OF *ACORUS CALAMUS* ON HUMAN NEUTROPHILS**

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#### ABSTRACT

*Acorus calamus* belongs to family Acoraceae. It has been used medicinally for a wide variety of ailments, and its aroma makes calamus essential oil valued in the perfume industry. Petroleum ether, chloroform, benzene, alcoholic and aqueous extracts of *Acorus calamus* leaves were tested for various phytoconstituents. The immunomodulatory activity was screened by *in-vitro* immunostimulant activity by phagocytic stimulation (slide method) and nitro-blue test. As per preliminary phytochemical investigation alcoholic extract showed the presence of flavonoids, tannins, triterpenoids, and proteins. From phagocytic stimulation test and nitro-blue tetrazolium test petroleum ether, alcoholic extract and volatile oil showed highly significant ( $p < 0.001$ ) at 5-15 mg/ml concentration. The results were comparable with control. The present study showed that *Acorus calamus* stimulate cell mediated immune system by modulating the neutrophil function.

**Keywords:** *Acorus calamus*, immunomodulatory activity, phagocytosis.

#### INTRODUCTION

Adaptogenic drugs that help an organism to cope better during stress and retard ageing process are well recognized in Ayurvedic medicine. Herbal drugs are known to have immunomodulatory property<sup>1</sup>. These immunomodulatory agents can act by stimulating both non specific and specific immune responses. These are useful for prevention or treatment of immunodeficiency related disorders like AIDS. Even in modern era a large extent of Indian population still relies on traditional system of medicine which are mostly plant based<sup>2</sup>. Hence it is considered necessary to experimental evidence to validate the traditional use of one such plant *Acorus calamus* (Acoraceae) is an erect branched herb glabrous<sup>3</sup>. Traditionally the plant is used as a pungent, coolant, anthelmintic, laxative, ulcer, leucoderma, uterine complaints. *Acorus calamus* is proved for anti-inflammatory, hepatoprotective, antibacterial, antiulcer, antioxidant<sup>14</sup>.

A literature survey reveals that no scientific study has been made with respect to immunomodulatory activity of *Acorus calamus*. Therefore in the present study *Acorus calamus* leaf extracts is reported to have immunomodulatory activity.

#### MATERIALS AND METHOD

##### Plant material

*Acorus calamus* Linn. fresh leaves were collected from local area of Jalgaon. Botanical identification was confirmed by Prof. Harshad N Pandit, Gurunanak Khalsa College, Department of Botany, Matunga, Mumbai.

##### Preparation of the extract

The shade dried powdered leaves of *Acorus calamus* was extracted by simple maceration method with petroleum ether (40-60), chloroform, benzene, alcohol and chloroform water I.P were used as solvents at room temperature for 7 days<sup>4</sup>. The extracts were filtered and concentrated at room temperature to avoid the decomposition of natural metabolites. Volatile compounds were isolated from plant material by hydro-distillation method 1998 BP the essential oils were separated from aqueous layer and dried over anhydrous sodium sulphate<sup>5</sup>. The extracts were chemically tested for the presence of different constituents by using standard method. The alcoholic extract showed the presence of flavonoids, tannins, triterpenoids, proteins. All extracts and volatile oil were subjected to the study for their immunostimulant activity using *in vitro* slide method and nitro-blue tetrazolium test on human neutrophil<sup>6,7</sup>.

##### Study of the immunostimulant Activity

##### *In vitro* Immunostimulant activity studies by slide method

###### Preparation of samples

Samples for *in vitro* study were prepared by dissolving 10 mg of extract and volatile oil in the 0.5 ml of Dimethyl sulphoxide (DMS) and diluting with normal saline. Concentration range was from 5, 10, 25, 50 and 100 mg/ml<sup>8</sup>.

###### Preparation of *Candida albicans* suspension

The *C. albicans* culture was incubated in the subgrades broth overnight. The *C. albicans* cells after overnight growth were centrifuged so that the cell button was formed at the bottom and supernatant was discarded. The cell button was then flooded with the sterile balanced salt solution and centrifuged again this was done

3-4 times the final cell bottom was flooded with a mixture of sterile balanced. Salt solution and Human serum in proportion of 16:4 cell were properly mixed in the vortex<sup>9,10</sup>.

#### Evaluation of phagocytosis

Human blood (2-3 drops) obtained by finger prick method was collected on the cotton pad in the sterile petri plate and incubated at 37°C for 25 minutes. After incubation, the clot was removed very gently, and the slide was slowly drained with sterile normal saline, taking care not to wash the adhered neutrophils (invisible)<sup>7,8</sup>. The slide was flooded with predetermined concentration of samples and incubated at 37°C for 15 minutes. The slide was then flooded with suspension of *C.albicans* and incubated at 37°C for 1 hour. The slide was drained fixed with methanol and stained with giemsa stain. The mean number of phagocytosed cells on the slide were determined microscopically for 100 granulocytes using morphological criteria. This number was taken as the phagocytic index (PI) and was compared with basal PI of controls. This procedure was compared with basal PI of controls<sup>7,8</sup>. This procedure was repeated for different concentrations of all extract and volatile oil as given in Table 1.

Immunostimulation in % was calculated by using following equation:-

$$\text{Stimulation} = \frac{(\text{PI}_{\text{test}} - \text{PI}_{\text{control}})}{\text{P.I.}_{\text{control}}} \times 100$$

#### Nitroblue Tetrazolium NBT Test

##### Preparation of Test Solution

Stock solution of in-vitro studies were prepared by dissolving petroleum ether, benzene, chloroform, alcoholic, aqueous extracts and *Acorus calamus* volatile oil in 0.5 ml of dimethyl sulphoxide (DMSO) and with phosphate buffer salt solution according to concentration range from 5 µg/ml, 10 µg/ml, 50 µg/ml and 100 µg/ml.

##### Evaluation of Nitroblue Tetrazolium Test

The leucocyte suspension (5 x 10<sup>6</sup>/ml) of 0.5 ml and 0.25 ml of freshly prepared Nitroblue tetrazolium (0.15%) were incubated at 37°C in an incubator for 20 min. After incubation the leucocyte

suspension centrifuged gently at 400 g for 3 minutes. The supernatant were discarded. The precipitate of cells were resuspended with phosphate buffer saline pH 7.2. The films were prepared on microscopic slide fix gently by heating and counterstain with dilute carbol fusion. Then slides were washed with running water. The percentage of Nitroblue tetrazolium positive cell counting blue deposition were counted. This procedure was repeated for different concentrations of the extracts and volatile oil as given in table 2. For control 0.25 ml of leucocyte suspension and 0.2ml of freshly prepared nitroblue tetrazolium (0.15%) were incubated<sup>16,17</sup>.

#### Statistical Analysis

Results of immunomodulatory activity were represented as Mean±SEM. Statistical analysis of data was performed using one way analysis of variance (ANOVA) was carried out followed by Dunnet's 't' test.

## RESULTS AND DISCUSSION

#### Evaluation of Phagocytosis

*Acorus calamus* inhibited phagocytosis of human neutrophils at concentration 100 µg/ml. Whereas stimulation of phagocytosis was observed in the concentration range 5 – 50 µg/ml in Alcoholic, petroleum ether extracts and volatile oil and found to statistical significance (p<0.001) when compared to control. The aqueous, chloroform, benzene extracts failed to show any effect on phagocytosis of granulocytes. This apparently inactive nature of extracts. The dose dependent switch of alcoholic and petroleum ether and volatile oil at 100 µg/ml. This behavior that is non-reactivity incertain concentration range with biphasic activity profile, the results are shown in table no.1.

#### Evaluation of Nitroblue Tetrazolium Test

The result obtained indicate that *Acorus calamus* leaves, volatile oil, petroleum ether and alcoholic extracts has stimulated the neutrophils phagocytic activity to the extant of 70% at concentration range 5 – 50 µg/ml were found to significant (p<0.001) compared to control.

**Table 1: Effect of *Acorus calamus*. Extracts and Volatile oil on Neutrophil Phagocytosis by Slide Method**

Concentration in µg/ml	Control	Aqueous Extract	Ethanollic Extract	Chloroform Extract	Benzene Extract	Pet. Ether Extract	<i>Acorus calamus</i> Oil
	Mean Percentage of Stimulation ± SEM						
5	7.37 ±0.91	5.92.00 ±0.57	10.66 ±1.08	7.00 ±1.05	5.39 ±0.32	11.73 ±0.32	12.41 ±1.20
10	7.37 ±0.91	8.00 ±1.23	12.29 ±1.15*	8.29 ±1.05	5.39 ±0.45	16.00 ±1.46*	18.22 ±2.18*
25	6.32 ±0.35	10.62 ±1.26	41.36 ±2.32*	12.28 ±2.69	8.36 ±0.56	32.18 ±2.30*	42.65 ±1.60*
50	6.32 ±0.35	8.20 ±2.10	45.32 ±2.54*	9.15 ±1.06	8.23 ±2.41	40.62 ±2.08*	45.00 ±1.45*
100	6.00 ±0.52	6.36 ±0.32	8.34 ±1.86	7.24 ±1.06	8.42 ±0.98	5.89 ±0.63	4.76 ±0.36

Significant difference from Control by one way ANOVA, followed by Dunnet's 't' test. \* p<0.001 Vs. Control; n=3.

**Table 2: Nitroblue tetrazolium NBT Qualitative Test**

Concentration in µg/ml	Control	Aqueous Extract	Ethanollic Extract	Chloroform Extract	Benzene Extract	Pet. Ether Extract	<i>Acorus calamus</i> volatile oil
	Mean Percentage Reduced Neutrophil ± SEM						
5	22.56 ±2.19	21.33 ±0.88	34.06 ±0.84	17.62 ±0.58	19.00 ±1.45	33.36 ±0.85	31.14 ±0.85
10	18.96 ±0.81	19.62 ±1.45	42.83 ±2.01*	25.31 ±1.20	14.93 ±1.85	35.83 ±0.83*	29.0 ±0.20*
25	19.60 ±0.52	23.43 ±1.67	53.20 ±1.68*	17.00 ±1.05	24.16 ±1.45	44.00 ±1.62*	48.36 ±2.01*
50	18.43 ±1.36	17.44 ±1.42	70.66 ±1.76*	25.23 ±0.88	15.33 ±1.76	65.00 ±2.30*	68.00 ±1.70*
100	18.66 ±0.86	22.32 ±0.24	17.00 ±1.73	20.66 ±1.76	16.66 ±2.02	23.87 ±1.33	16.00 ±0.52

Significant difference from Control by one way ANOVA followed by Dunnet's 't' test.

\* p<0.001 Vs. Control; n=3.

However at high concentration 100 µg/ml fail to stimulate phagocytic activity the dose dependent switch of volatile oil and both extract, the aqueous, chloroform, benzene extract fail to stimulate neutrophils. The results are shown in Table 2.

Immunomodulatory agent of plant origin enhance the immune responsiveness of an organism against a pathogen by activating the immune system. However *Acorus calamus* extract can be subjected to systematic studies to substantiate the therapeutic claim made with regard to their clinical utility.

## DISCUSSION

In present study petroleum ether, alcoholic extracts and volatile oil exert general immunostimulation effect in both phagocytosis of candida and nitroblue tetrazolium test at the concentration range 5 – 50 µg/ml concentration on human neutrophils<sup>11,16</sup>. The immunostimulant activity may be due to the presence of flavonoids, tannins, carbohydrate and proteins present in alcoholic extract and volatile oil principle present in volatile oil, and petroleum ether extract. However it need further investigation.

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