Research Article

Chemical Composition and Anti-Inflammatory Potential of Essential Oil of *Alpinia calcarata* ROSC. - grown in South India.

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ABSTRACT

Alpinia calcarata Rosc. is an aromatic medicinal plant belong to Zingiberaceae family, its rhizome is used in traditional medicine to cure lot of diseases like cold, asthma, bronchitis, stomach ache, fever, throat inflammation and rheumatism. This present study examines the chemical composition and its *invivo* anti-inflammatory activity of essential oil of *A.calcarata* cultivated in western ghats of South India. The chemical composition of essential oil of *A. calcarata* was analyzed by GC-MS: 22components were identified and the major compounds of essential oil of *A. calcarata* were 1, 8-cineole (17.72%), camphor (11.71%), α-myrcene (10.38%) and Borneol (6.29%). The *in vivo* anti-inflammatory activity of essential oil was evaluated by carrageenan induced paw edema model in albino wistar rats. The essential oil was able to inhibit *in vivo* anti-inflammatory about 75.78% and 78.15%at 200mg/kg and 300 mg/kg (Paw edema method)compared to standard indomethacin produced 79.58% at 10mg/kg for 5h period. The results indicate that *A.calcarata leaves* essential oil showed significant anti-inflammatory activity compared to standard employed. This result is the scientific evidence as *A.calcarata* used in Indian folk medicine.

Keywords: *Alpinia calcarata*, GC-MS, 1, 8-cineole, camphor, α-myrcene and *invivo*anti inflammatory.

INTRODUCTION

Inflammation is the positive response of our body to resist the changes in the defense system by infectants or tissue breakdown, enzyme activation and cell migration. Recently much research has been paying attention in the search of medicinal plants and crude drugs with anti-inflammatory potential which may lead to the discovery of new plant based medicine to suppress the inflammation and reduce the cost and side effects of the synthetic drugs like NSAID which are used against inflammation. In this context every possible medicinal plant has tested for anti-inflammatory activity which is used in traditional medicine.

Alpinia calcarata Rosc.is economically important aromatic medicinal plant belongs to the family *Zingiberaceae* distributed in India, Myanmar, Indonesia, Thailand and Sri Lanka[1]. The rhizomes are medicinally important part and effective against different diseases like cold, asthma, bronchitis, throat inflammation[2]. It is traditionally used to treat rheumatism, diabetes, and fever and stomach ache[3]. In Sri Lanka *A.calcarata* is commonly prescribed to treat arthritis, cough, respiratory ailments and diabetes[4,5]. Some diterpenes such as calcaratarins A-E, sesquiterpenes such as shyobunone and coumarins such as herniarin from the rhizomes of *Alpinia calcarata* grown in China was previously isolated[6]. Moreover,some benzenoids such as protocatechuic acid, vanillic acid andsyringic acid, terpenoids, phenolic compounds, flavonoids and alkaloids were isolated from the leaves of *A.calcarata* grown in India[7]. In recent years the pharmacological potential of *A.calcarata* was evaluated by many researchers. It showed antibacterial, antifungal, antioxidant, anti-diabetic, cytotoxic and anti-inflammatory activites[8-11]. There are many reports available for the chemical composition of essential oil, 1; 8-Cineole had been found to be the major constituent in theoil[6, 7, 12, 13]. 1, 8-Cineole has been demonstrated to be capable of reducing inflammation and pain[9]. However, anti-inflammatory activity of leaves of *A.calcarata* essential oil has not been reported. So the present study deals with the investigation of the chemical components of essential oil of *A.calcarata* leaves and its anti-inflammatory potential using animal models.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *A.calcarata* were collected from home garden in Pollachi between the period of June –July. The plant material was identified and authenticated by Department of Botany, NGM College, Pollachi, Coimbatore and Tamilnadu. The voucher specimen (16CHE002) was preserved in the Chemistry department.

Isolation of essential oil

About 2kg of fresh leaves was subjected to hydro distillation using Clevenger type apparatus for 3h. The oil obtained was dried over anhydrous sodium sulphate and stored in a container and kept in freezer until GC-MS analysis.

GC-MS analysis

The essential oil from leaves of *A.calcarata* was analyzed by GC–MS using thermo GC - trace ultra version: 5.0 coupled with thermo MS DSQ II instrument. Compounds were separated on DB-35, fused silica MS capillary standard non - polar column (30m x 0.25 mm), film

thickness 0.25 µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 70̊C and held for 2 minutes and the temperature of the oven was raised to 260̊C for 10min and raised 6̊C per minute and final temperature was 300̊C for 10min. The sample of 100 µL was dissolved in 1mL of acetone and injected with split less mode. Mass spectra were recorded over 50–500 amu range with electron impact ionization energy 70 eV, while injector and MS transfer line temperature were set at 230˚ C and 280˚ C respectively.

Identification of essential constituents

The components were identified by comparison of their mass spectra with those of the NIST mass spectral library ver.2.0d, as well as on comparison of their retention time either with those of authentic compounds or with literature values.

Animals and approval

Wistar Albino rats weighed around 150-175g of either sex were purchased and used for this study. They were housed in the animal house at the temperature of 25 - 27˚C, the relative humidity of 50-60% under 12 h light and dark cycles was maintained. The animals had free access to food pellets and water was given ad libitum. The experiment was conducted according to Institutional animal ethics committee (IAEC), India (CPCSEA /030/2015).

***In-vivo* Anti Inflammatory Activity**

Carrageenan Induced Rat Paw edema method

This method was carriedout by the procedure described by winter et al[14]. Animals were divided into 4 groups of six animals each. Before treatment, the volume of the right paw of each animal was measured using a digital plethysmometer. The group I received control vehicle orally. Group II animals received the standard Indomethacin (10mg/Kg p.o) and Group III and Group IV received essential oil in two different doses 200mg/kg and 300mg/kg. After 30min the rats were challenged with subcutaneous injection of 0.1ml of 1%w/v solution of carrageenan into sub plantar region of left paw. The paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to the mark. The paw volume was measured at 1h, 2h, 3h, 4h and 5h after carrageenan injection in control, essential oil treated and standard Indomethacin treated groups using digital plethysmometer. The difference between initial and subsequent reading gave the actual edema volume.

% Inhibition= Vc-Vt/ Vc X 100
V_c– Mean change in Paw edema volume of control groups
V_t– Mean change in Paw edema volume of essential oil treated and standard groups

Toxicity studies

The toxicity study was carried out as per OECD guideline-425 on albino rats of three groups with 4 animals in each group. The essential oil was given orally at various dose levels of 100mg/kg, 200 mg/kg, 1g/kg and 1.5g/kg body weight after one day fasting. The rats were observed closely for changes and abnormalities if any produced for first three hrs. The continuous observation was made regularly for24 hr.

Statistical analysis

The experiment was conducted three times for independent results and was expressed as means ± standard. Analysis of variance was evaluated by one way ANNOVA method. Significant differences in ($^{*}P$ <0.05 and $^{**}P$ <0.01) were analyzed by Dunnett's test.

RESULTS

A.calcarata essential oil was greenish yellow in color, about 0.95 % w/v of yield. The oil was analyzed by GC-MS.The results were shown in Table I. and GC-MS chromatogram was given in figure 1. GC-MS analysis gave 22 components representing 94% of the essential oil composition. The major essential oil composition was1,8-cineole (17.72%), camphor (11.71%), α- myrcene (10.38%) and Methyl 2-(phenoxysulfonyl) ethanimidoate (9.76), other important components are borneal (6.29%), linalyl propionate (4.97%), endobornyl acetate (4.34%), phytol (1.48%) and Himachalol (2.74%).

Table 1: Chemical composition of essential oil of A.calcarata

S.No	Name of the compound	R.T	Percentage (%)
1.	Methyl 2-(phenoxysulfonyl) ethanimidoate	3.55	9.76
2.	a- Myrcene	4.48	10.38
3.	1,8-Cineole	5.57	17.72
4.	Linalool	6.49	0.83
5.	a-Bisabolene	7.75	0.29
6.	Camphor	8.13	11.71
7.	Linalyl propionate	8.46	4.97
8.	Myrtenal	8.93	0.66
9.	Endobornyl acetate	9.53	4.34
10.	5-Phenylisothiazote	10.27	5.27
11.	Streptazone B2	11.41	5.37
12.	Borneal	12.89	6.29
13.	a-Chamigrene	13.50	1.77
14.	Carotol	16.17	7.79
15.	a-Cedrol	17.45	0.71
16.	Farnesol 2	17.92	0.45
17.	Himachalol	18.30	2.74
18.	Longiborneol	20.28	0.60
19.	Phytol	25.24	1.48
20.	4,5-Methanochrysene	26.60	1.08
21.	9-Cyclohexylnonadecane	29.46	0.27
22.	1,12-Tridecadiene	30.03	0.32

Acute toxicity studies

In this study the essential oil of *A.calcarata* showed no mortality even at higher dose levels to the tested rats (1g/kg and 1.5g/kg). Hence the dose level 200mg/kg and 300g/kg were used for *invivo* anti-inflammatory study.

***In-vivo* anti-inflammatory activity**

Carrageenan induce hind paw edema method

There is no change in the paw volume of rats in the carrageenan control group. The essential oil added groups showed significant inhibitory effects on hind paw edema induced by carrageenan at the dose level of 200mg/kg and 300mg/kg body weight gave 75.78% and 78.17% respectively for fifth hour. The standard drug Indomethacin 10mg/kg body weight showed 79.58% of inhibition given in table II.

Table 2: *In-vivo* anti-inflammatory activity of *A.calcarata* by carrageenan induced hind Paw edema method.

Treatments	Paw edema volume										
	1hr		2hr		3hr		4hr		5hr		% inhibition 5hr
	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	
Only arthritis/Control	1.219207	0.001396724	1.179207	0.001396724	1.027873	0.001215514	1.043207	0.001396724	1.016507	0.003336275	-
Standard	1.191607	0.004970674	0.888907*	0.004970667	0.582573 4**	0.003657982	0.33254**	0.004866179	0.2075067**	0.004113896	79.58629896
Essential oil 200mg/kg	1.205307	0.004113892	0.909207*	0.004862858	0.600706 6*	0.004113909	0.3450733**	0.003524793	0.2461733**	0.003657982	75.78242944
Essential oil 300mg/kg	1.236073	0.003657982	0.892207*	0.004113893	0.552473 4*	0.004827	0.3383067**	0.003336294	0.2220733**	0.004782691	78.15329358

(Values are expressed as Mean ± SEM; n= 6; *P<0.05; **P<0.01)

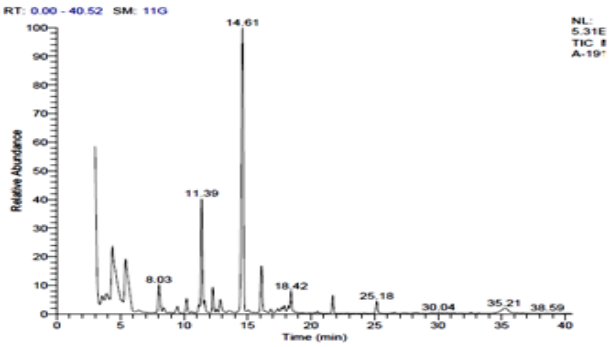

Figure 1: GC-MS chromatogram of essential oil of *A.calcarata*

DISCUSSION

The chemical components of essential oil of *A.calcarara* leaves were analyzed by GC-MS chromatogram. The essential oil yield of the plant was 0.95%w/v and 22 components are identified and mostly terpeneoids are present. 1, 8 cineole, camphor, phytol, fenchyl acetate and linalool was the important major compounds in literature. In Sri Lanka *A.calcaata* is extensively used in traditional medicine and essential oil showed 1,8-cineole and fenchyl acetate was the major compounds[1]. The rhizome oil constituents from Bangladesh containing Eucalyptol, camphene β-mycene, tepinyl acetate, fenchyl acetate and methyl cinnamate was the major components[10, 15]. Indian oil showed 1,8- cineole, fenchyl acetate, camphor, borneal was major compounds[12,16-19]. Rhizome essential oil contains fenchyl acetate along with 1,8 cineole as the major compounds while fenchyl acetate was absent in the leaf essential oil.

Carrageenan induced inflammation is the most sensitive, widely used method for evaluating anti-inflammatory activity. It is a good inflammating agent for screening the natural products for their anti-inflammatory potential[20]. The time course of the increase in paw edema volume induced by cargeenan is biphasic event, development of inflammation in the firstphase is due to release of histamine, serotonin and other related substances[21]. The second phase and third phase are associated with the activation of Kinin like substances and the release of prostaglandins proteases and lysomes[22]. Essential oil of *A.calcarata* leaves showed reduction in inflammation in rat hind paw when challenged with carrageenan induced inflammation. The significant (*P <0.05, **P< 0.01) anti-inflammatory property was observed in 2h,3h, 4h and 5hrs treatment of essential oil reduced the paw edema. The oil showed dose dependent activity against inflammation, the % inhibition of essential oil of 200mg/kg, 300mg/kg and standard Indomethacin 10mg/kg dose level was 75.78%, 78.17% and 79.58% respectively for 5 hrs time duration. The results are comparable with standard drug employed. The % of inhibition at 300mg/kg dose level was 78.17% which is nearly same that of the % of the inhibition of the standard drug at 10mg/kg. Generally the plant based products are used for arthritis, reducing pain and inflammation has anti-inflammatory properties. However, the scientific validity of anti-

inflammatory activity of *A.calcarata* leaves essential oil was not investigated so far. This is the first kind of report for anti-inflammatory activity of *A.calcarata* essential oil grown in the Western ghat region, Tamilnadu. Only two reports are available for anti-inflammatory properties of *A.calcarata* essential oil and the % of inhibition was 52.3% at 380mg/kg dose level[10]. Hot water extract and hot ethanol extract of *A.calcarata* rhizomes were evaluated in Sri Lanka, at 500mg/kg of ethanol extract showed superior inhibition than the standard drug at 4hr[23]. Our findings also have a similar result which is due to the presence of triterpenoids which impairs histamine release from mast cells and exerts anti-inflammatory effect[24].

CONCLUSION

The chemical composition of essential oil of *A.calcarata* was evaluated by GC-MS, 1,8 cineole, camphor and α-myrcene was the major compounds. The oil was tested for the *invivo* anti-inflammatory effect by carrageenan induced hind paw edema model. It showed significant reduction in inflammation. This might be due to the presence of 1, 8-cineole and camphor present in the oil. This finding gave scientific support to the plant which is used to treat the inflammation, swelling and pain in traditional medicine.

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