Journal of Spices and Aromatic Crops
Vol. 32 (2): 202-213 (2023)
10.25081/josac.2023.v32.i2.8876

Indian Society for Spices

# Morphological and biochemical characterization of *Alpinia calcarata* rhizomes

K Shibila[1*], A K Aneesha[1], A Suma[2], P Anitha[1] & C R Rashmi[3]
[1]*Department of Plantation, Spices, Medicinal and Aromatic crops, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur – 680656, Kerala.*
[2]*ICAR NBPGR Regional Station, Thrissur - 680656, Kerala.*
[3]*AICVIP, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur - 680656, Kerala.*
**Email: shibila-2020-12-010@student.kau.in*

Received 24 February 2023; Revised 28 February 2024; Accepted 04 March 2024

## Abstract

Lesser galangal rhizomes are well known for their anti-inflammatory, anti-microbial and anti-oxidant properties. The aim of the present study was to assess the variability in morphological and biochemical characters of lesser galangal, *Alpinia calcarata* accessions collected from various South Indian states. The results revealed that accession IC373608 was significantly superior with respect to yield contributing traits including the rhizome length, width, fresh yield and dry recovery (6.02 cm, 2.40 cm, 14.63 g tiller$^{-1}$ and 33.26% respectively) among the eighteen accessions studied. The biochemical analysis of rhizomes revealed that the maximum volatile oil and oleoresin content were found in the accession IC468880 (0.75%) and IC373608 (34.20%) respectively. The highest values for total phenols, total flavonoids and starch were observed in the accessions IC373608 (115.25 mg GAE.g$^{-1}$), IC582825 (55.65 mg QE. g$^{-1}$) and IC210656 (44.05 mg. g$^{-1}$) respectively. The accession IC550112 (7.90%) recorded the lowest crude fibre content, whereas the highest total terpenoid content was noticed in accession IC210421 (20.25%). The GC MSMS profiling of volatile oil of *A. calcarata* rhizome showed the presence of 28 compounds in it. Considering all economically important characters, accessions IC373608, IC582825 and IC210421 were found to be superior and can be utilized in future crop improvement programmes.

**Keywords:** *Alpinia calcarata*, biochemical characters, GC MSMS analysis, rhizome, variability

## Introduction

*Alpinia calcarata* Rosc. (Family: Zingibercaeae), commonly known as lesser galangal in English and Rasna in Sanskrit, is a widespread rhizomatous perennial herb, used in traditional medicinal systems. It is cultivated throughout the tropical and subtropical Asian countries including India, Bangladesh, Sri Lanka, and Malaysia. It is a perennial herb growing up to a height of 100-120 cm. Leaves are simple, alternate, dark green in color with linear lanceolate shape of 40-55 cm long and 2.5-5.0 cm broad. The inflorescence is a terminal panicle. Flowers are irregular, bisexual and pedunculate having creamy yellow colour with red streak. Mature rhizomes are cylindrical, less branched, bearing numerous fibrous root tubers.

The economic part of *A. calcarata* is the rhizomes which are the major constituents of many formulations in the indigenous system of medicine for relieving throat inflammation, stimulating digestion, purifying blood and improving the voice. The rhizome extract is widely used to treat cough, respiratory ailments, asthma, arthritis, diabetes, rheumatism, bronchial catarrh and for reducing pain (Jayaweera, 1981). It is an important constituent of the polyherbal formulations like Rasnadi churnam, Rasnasavam, Maharasnadikashayam, Rasna saptakam, etc. The plant also possesses several pharmacological properties like anti-oxidant, anti-ulceric, anti-spasmodic, anti-microbial, gastro-protective and anti-diabetic (Khandel *et al*., 2018).

A wide range of volatile constituents are present in the essential oil of *A. calcarata* and major compounds are eucalyptol, ethyl iso-allocholate, $\alpha$-fenchyl acetate, camphor (Bhuiyan *et al*., 2011). The rhizomes of *A. calcarata* are very rich in terpenoids, sterols, phenols, flavonoids alkaloids, tannin, saponins, proteins and carbohydrates (Raj *et al*., 2011). There are only a few reports on volatile oil and biochemical composition of the rhizomes. However, there are no comprehensive studies on variability including morphological and biochemical characters on *A. calcarata.* Hence, the present study was carried out to assess the variability in morphological and biochemical characters of *A. calcarata* accessions which had been collected from various South Indian states and also to identify elite genotypes with desirable traits for further crop improvement programme.

## Material and methods

The present investigation was undertaken at the Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur and ICAR - NBPGR Regional Station, Thrissur during 2021-2022. The study was performed in the existing plant population of seventeen accessions of *A. calcarata* collected from South Indian states *viz.* Kerala, Tamil Nadu and Karnataka which are maintained at ICAR - NBPGR Regional Station, Thrissur and one accession maintained at Plantation farm, under Department of Plantation, Spices, Medicinal and Aromatic crops, College of Agriculture, Vellanikkara. After harvesting the plant, observations on the

rhizome characters *viz.* rhizome length, width, yield and dry recovery were recorded. The colour of outer skin and inner core were assessed using the latest Royal Horticultural Society (RHS) colour charts. Analysis of variance was performed using SPSS version 22 software.

**Biochemical characters of rhizome**

The standard biochemical methods were adopted to estimate volatile oil, oleoresin, total phenols, total flavonoids, starch, crude fibre and total terpenoids content in the rhizomes. Results were statistically analysed using ANOVA and the design followed was completely randomized design (CRD) with two replications.

**Volatile oil**

The volatile oil content in the dried rhizome was determined by hydro distillation method using Clevenger's apparatus (Guenther, 1972). After washing and cleaning, the rhizomes were chopped into small pieces and dried. The dried rhizome (100g) was taken in a round bottom flask and distilled water was added. The flask was fitted with Clevenger's apparatus and kept on a heating mantle for 5 to 6 hours. The oil that was formed in the uppermost layer was collected in eppendorf tubes. It was calculated using following formula.

$$\text{Volatile oil (\%)} = \frac{\text{Volume of oil (ml)}}{\text{Weight of sample (g)}} \times 100$$

**Oleoresin**

The oleoresin in the dried rhizomes was determined by solvent extraction method using Soxhlet apparatus. Ten grams of dried rhizome powder was taken in thimble and kept in Soxhlet extractor. Then 250 ml methanol was added to the flask and the extraction apparatus was assembled. The extraction was carried out in a water bath. When the solvent became colourless, the extract was collected, desolvated and weighed. The oleoresin content in the sample was calculated as

$$\text{Oleoresin content (\%)} = \frac{\text{Weight of residue (g)}}{\text{Weight of sample (g)}} \times 100$$

**Total phenols**

The total phenols in the rhizome were measured based on the Folin -Ciocalteu method (Mallik and Singh, 1980). One mg of dried rhizome sample was taken and homogenized with 10 ml of 80 per cent ethanol using mortar and pestle. The supernatant was saved after centrifugation. One ml of the rhizome extract was diluted with 7.5 ml of water. To the extract, 1 ml of Folin-Ciocalteau reagent and 800µl of $Na_2CO_3$ (20%) were added and incubated for 2 hours in dark at room temperature. The absorbance was read at 725 nm using Spectrophotometer. The result was expressed in mg gallic acid equivalent $g^{-1}$.

**Total flavonoids**

The total flavonoids were estimated based on the method described by Sareena (2011). The rhizome sample (5 mg) was weighed and homogenized with 80 per cent methanol. It was centrifuged at 10000 rpm for 20 minutes. The supernatant was collected, the process was repeated 2-3

times, supernatants were pooled and the volume was made up to 50 ml. To one ml sample aliquot 0.3 ml $NaNO_2$ (5%), 0.3 ml $AlCl_3$ (10%) and 2.4 ml NaOH (1N) were added and incubated for 10 minutes at room temperature. Later, the absorbance was read at 510 nm. The result was expressed in mg quercetin equivalent $g^{-1}$.

**Starch**

The starch content was estimated by Anthrone method (Hedge and Hofreiter, 1962). The dried rhizome sample (0.1 g) was homogenized in hot ethanol (80%) to remove sugars and the sample was extracted with perchloric acid (52%). To one ml aliquot of the sample, 4 ml of anthrone reagent was added. The mixture was heated for 8 minutes. After cooling, the intensity of green to dark green colour was read at 630 nm in a spectrophotometer. The result was expressed in mg $g^{-1}$ dry weight.

**Crude fibre**

The crude fibre content was estimated by the method described by Sadasivam and Manickam (1992). Two grams of dried rhizome powder was weighed into a conical flask and boiled with 200 ml of sulphuric acid for 30 minutes. Then filtered using muslin cloth and washed with boiling water to remove acid residue completely. The filtrate was again boiled with 200 ml of sodium hydroxide solution for 30 minutes. The sample was filtered through muslin cloth and washed using boiling water until washings were no longer alkaline. The residue was collected in a pre-weighed ashing dish (W1) and dried in hot air oven at a temperature of $230^0$C for two hours. The dish was cooled in a desiccator and weighed (W2) and ignited in a muffle furnace for 30 minutes at $600^0$C. The dish was cooled and reweighed (W3). The result was expressed in percentage. The crude fibre percentage was calculated using the following formula.

$$\text{Crude fibre content (\%)} = \frac{\text{Loss in weight on ignition (W2-W1) - (W3-W1)}}{\text{Weight of the sample}} \times 100$$

**Total terpenoids**

The total terpenoid content was analysed using Ferguson method (Ferguson, 1956). 100 mg dried rhizome powder was taken and soaked in 9 ml of alcohol (95% ethanol) for 24 hours, filtered and the filtrate was extracted with 10 ml of petroleum ether using separating funnel. The ether extract was collected in pre-weighed glass vials and allowed for its complete drying. The ether extract was treated as total terpenoids. The percentage of total terpenoids was calculated using the following formula.

$$\text{Total terpenoid content (\%)} = \frac{\text{Weight of terpenoid extract (g)}}{\text{Weight of sample (g)}} \times 100$$

**GC MSMS profile of volatile oil**

GC MSMS profile of volatile oil was analysed from *A. calcarata* accession IC373608 which was found superior in yield and quality traits. The volatile oil of rhizome was analysed by Triple quadruple GC MSMS (Model TSQ 8000 MSMS). TG5M5 column of 30 mm x 0.25 mm x 0.25 μm dimension was used as stationary phase. The oven temperature increased from $60^0$C to $240^0$C at a constant rate of $3^0$C/min.

Helium was used as carrier gas at a flow rate of 1 ml/min. One microliter of the essential oil was injected by Finnigan Autoinjector A13000 with split ratio of 10:1. MS was conducted by electron impact positive mode at 70 electron volts. The chemical constituents were analysed by comparing mass spectra and retention time indices with NIST MS Search 2.0 Library. Peak area was expressed in percentage.

## Results and discussion

### Rhizome characters

Data pertaining to rhizome characters including rhizome length, width, yield and dry recovery is presented in the Table 1.

Since rhizomes are the economic part of *A. calcarata*, rhizome dimension has a great impact on yield. Accession IC373608 recorded higher value for rhizome length (6.02 cm) and which on par with all other accessions except IC468880 and IC582756. Significantly higher value for rhizome width was noted in the accession IC373608 (2.40 cm) and it was on par with IC582825 (2.20 cm). Mathew *et al*. (2014) observed the fresh mean rhizome length of *A. calcarata* as 2.0 to 6.0 cm and mean width as 2.0 cm. In *A. galanga*, the mean rhizome length was 6.0 to 7.0 cm and width was 2.0 to 4.5 cm (Trimanto *et al*., 2021)

Rhizome yield is the important economic trait which decides the superiority of an accession. In the present study, rhizome yield varied significantly among the accessions. The rhizome yield showed on par values in accession IC373608 (14.63 g tiller$^{-1}$) and IC582825 (13.47 g tiller$^{-1}$). Ponmozhi and Kalaiselvi (2011) had reported mean rhizome weight of 24.54 and 28.87 g tiller$^{-1}$ for *A. calcarata* and *A. officinarum* respectively. The dry recovery of rhizomes also varied significantly among the accessions and it ranged from 19.82 to 33.26 per cent. The highest dry recovery was observed in accession IC373608 (33.26%) followed by IC210421 (32.88%). Previous studies reported the dry recovery of *A. calcarata* rhizome as 25 per cent (KAU, 2016).

### Qualitative morphological rhizome characters

There was no variation in the qualitative morphological parameters of rhizomes among the accessions under investigation. The cylindrical rhizomes were less branched, bearing numerous root tubers. The skin colour of the rhizome was pale yellow (RHS 158A) and inner core was moderate yellow in colour (RHS 161A). Mathew *et al.* (2014) described *Alpinia calcarata* rhizomes with cylindrical shape and creamy yellow colour. Namdeo and Kale (2015) observed that the rhizome of *Alpinia galanga* was cylindrical in shape, reddish brown colour externally and orange yellow colour.

**Table 1.** Rhizome characters of *Alpinia calcarata* accessions

| Accession | Rhizome length (cm) | Rhizome width (cm) | Rhizome yield (g tiller$^{-1}$) | Dry recovery (%) |
|---|---|---|---|---|
| IC210421 | 5.64 | 1.72 | 12.68 | 32.88 |
| IC210656 | 5.52 | 1.76 | 11.82 | 28.65 |
| IC373608 | 6.02 | 2.40 | 14.63 | 33.26 |
| IC468880 | 5.28 | 1.80 | 12.03 | 25.19 |
| IC550112 | 5.68 | 1.76 | 11.98 | 26.26 |
| IC552519 | 5.62 | 1.80 | 11.60 | 26.8 |
| IC565484 | 5.82 | 1.02 | 10.19 | 20.33 |
| IC565495 | 5.66 | 1.28 | 10.83 | 19.82 |
| IC582756 | 4.98 | 1.24 | 9.74 | 22.77 |
| IC582781 | 5.66 | 1.70 | 11.06 | 21.76 |
| IC582783 | 5.84 | 1.36 | 11.30 | 25.59 |
| IC582788 | 5.46 | 1.58 | 10.28 | 20.04 |
| IC582800 | 5.44 | 1.48 | 11.12 | 25.62 |
| IC582809 | 5.60 | 1.62 | 10.63 | 20.43 |
| IC582819 | 5.64 | 1.70 | 12.39 | 30.61 |
| IC582825 | 5.94 | 2.20 | 13.47 | 31.04 |
| IC582826 | 5.80 | 1.65 | 12.95 | 27.54 |
| PCSAc 1 | 5.52 | 1.70 | 9.74 | 19.90 |
| Mean | 5.61 | 1.70 | 11.58 | 25.47 |
| CV (%) | 8.50 | 19.50 | 15.13 | 17.90 |
| CD (0.05) | 0.69 | 0.37 | 1.93 | NA (unreplicated) |

**Biochemical characters of rhizome**

The biochemical analysis of rhizomes showed significant variation in volatile oil, oleoresin, total phenols, total flavonoids, starch, crude fibre and total terpenoid content and is represented in the Table 2.

**Volatile oil (%)**

In the present investigation, volatile oil recovery from dried rhizomes ranged from 0.30 to 0.75 per cent. The highest oil recovery was recorded in the accession IC468880 (0.75%) and the lowest in IC565495 (0.30%). Raina and Abraham (2015) reported volatile oil content of 0.29 to 0.96 per cent from the dried rhizome of *A. calcarata* and IC373608 showed the highest volatile oil of (0.96%) and lowest in IC565495 (0.29%). Nampoothiri *et al.* (2016) also confirmed that the volatile oil content in dry rhizomes was 0.93 per cent.

**Oleoresin (%)**

The oleoresin content in rhizomes varied from 20.20 to 34.20 per cent. The highest oleoresin was recorded in IC373608 (34.20%)

and the lowest in accession IC565484 (20.20%). Arambewela and Arawwawala (2010) reported oleoresin content of 18.5 per cent in dried rhizome of *A. calcarata*. Similar results were also reported by Raj *et al.* (2011).

**Total phenols (mg gallic acid (GAE). $g^{-1}$)**

Significantly higher total phenols was recorded in the accession IC373608 (115.25 mg GAE.$g^{-1}$) and it was on par with IC210656 (112.80 mg GAE.$g^{-1}$). The present study corroborated the results of earlier findings. Ramya *et al.* (2015) reported that the total phenolic content in ethanolic extract of *A. calcarata* was 100.7 mg GAE. $g^{-1}$. Acetone extract of *A. calcarata* rhizome contained the highest phenol concentration (124.6 mg GAE.$g^{-1}$) followed by methanolic extract (115.95 mg GAE.$g^{-1}$) and the lowest in aqueous extract (25.2 mg GAE.$g^{-1}$) (Jisha *et al.,* 2021).

**Total flavonoids (mg quercetin (QE). $g^{-1}$)**

The total flavonoids ranged from 30.30 to 55.65 mg QE.$g^{-1}$ and the higher values of total flavonoids were observed in IC582825 (55.65 mg QE.$g^{-1}$), IC373608 (54.45 mg QE.$g^{-1}$), which were on par. Ramya *et al.* (2015) reported that the ethanolic extract of *A. calcarata* rhizome yielded flavonoid content of 24.2 mg QE.$g^{-1}$ while Singh *et al.* (2020) revealed total flavonoid content in rhizome extract was 36.92 mg QE.$g^{-1}$.

**Starch (mg.$g^{-1}$)**

The highest starch content was observed in IC210656 (44.05 mg.$g^{-1}$) and the minimum in IC582756 (27.65 mg.$g^{-1}$), IC565495 (27.75 mg.$g^{-1}$) and IC565484 (28.45 mg.$g^{-1}$), which were on par. Ponmozhi and Kalaiselvi (2011) also observed that that the starch content in the rhizome of *A. calcarata* was 38.2 mg.$g^{-1}$ and in *A. galanga*, it was 93.1 mg.$g^{-1}$. The rhizome powder of *A. calcarata* consisted of plenty of simple and compound starch grains and most of them were oval and round shaped but some of them were triangular and pear shaped (Mathew *et al.,* 2014).

**Crude fibre (%)**

Low crude fibre content is considered as a desirable character and the accession IC550112 (7.90%) recorded the lowest crude fibre. Accessions IC552519 (8.60%), IC373608 (8.70%), IC210656 (8.80%) and IC582819 (9.02%) were on par with IC550112. Indrayan *et al.* (2009) evaluated the nutritive value of rhizomes of certain ginger like species and found that the lowest crude fibre percentage was present in *A. calcarata* (7.25%). Nohir *et al.* (2016) recorded the crude fibre content in dried rhizome of *A. galanga* (9.86 per cent), while Afra and Ghannam (2022) recorded crude fibre (14.00%) of rhizomes on dry weight basis.

**Total terpenoids (%)**

Among the eighteen accessions, the total terpenoids ranged from 6.70 to 20.25 per cent in the rhizomes. The accessions IC210421 (20.25%), IC373608 (19.50%) and IC550112 (19.00%) showed the higher value of total terpenoids compared to other accessions and were on par. The rhizomes of *A. calcarata* were very rich in terpenoids, sterols, phenols, flavonoids, *etc.* (Raj *et al.,* 2011). Singh *et al.* (2015) reported total terpenoid content of 5.89 per cent in the

dried rhizome of *Curcuma amada,* while Datta *et al.* (2018) observed the total terpenoids of 12.36 $mg.g^{-1}$ in turmeric.

## GC MSMS profile of *A. calcarata* volatile oil

GC MSMS profile of volatile oil extracted from rhizome of *A. calcarata* accession IC373608 which was found superior in yield and quality traits is presented in the Table 3 and figure 1.

A total of 28 compounds were identified from volatile oil of *A. calcarata* rhizome. Eucalyptol (1,8-cineole) (19.17%) and ethyl iso-allocholate (15.06%) were found to be major components. Similar study was conducted by Tewari *et al.* (1999) who recorded 31 constituents in rhizome oil of *A. calcarata* and l,8-cineole (41.4%) was the major constituent. Arambewela *et al.* (2005) identified 18 compounds in rhizome oil of *A. calcarata* and 1,8-cineole (24.70%) was major component. Raina and Abraham (2015) identified main constituents of rhizome oil of *A. calcarata* germplasm as that 1,8-cineole, $\alpha$-fenchyl acetate, $\alpha$-terpineol, camphor, terpinen-4-ol and borneol.

**Table 2.** Biochemical characters of *Alpinia calcarata* accessions

| Accession | Volatile oil (%) | Oleoresin (%) | Total phenols (mg GAE.$g^{-1}$) | Total flavonoids (mg QE.$g^{-1}$) | Starch (mg.$g^{-1}$) | Crude fibre (%) | Total terpenoids (%) |
|---|---|---|---|---|---|---|---|
| IC210421 | $0.72^{b}$ | $27.80^{bcd}$ | $110.75^{bc}$ | $50.75^{ab}$ | $40.80^{c}$ | $9.70^{cd}$ | $20.25^{a}$ |
| IC210656 | $0.72^{b}$ | $24.80^{efgh}$ | $112.80^{ab}$ | $48.50^{abc}$ | $44.05^{a}$ | $8.80^{de}$ | $17.40^{ab}$ |
| IC373608 | $0.70^{b}$ | $34.20^{a}$ | $115.25^{a}$ | $54.45^{a}$ | $43.00^{b}$ | $8.70^{de}$ | $19.50^{a}$ |
| IC468880 | $0.75^{a}$ | $25.80^{def}$ | $95.20^{fgh}$ | $47.10^{bc}$ | $37.65^{e}$ | $10.42^{c}$ | $13.95^{bcd}$ |
| IC550112 | $0.58^{e}$ | $22.10^{ijk}$ | $107.90^{cd}$ | $43.60^{bcde}$ | $38.55^{de}$ | $7.90^{e}$ | $19.00^{a}$ |
| IC552519 | $0.48^{g}$ | $24.10^{fghi}$ | $102.75^{e}$ | $42.35^{cde}$ | $36.05^{f}$ | $8.60^{de}$ | $13.65^{bcd}$ |
| IC565484 | $0.34^{ij}$ | $20.20^{k}$ | $91.50^{i}$ | $34.30^{fg}$ | $28.45^{k}$ | $14.50^{a}$ | $6.70^{g}$ |
| IC565495 | $0.30^{k}$ | $28.20^{bc}$ | $92.75^{hi}$ | $30.40^{g}$ | $27.75^{k}$ | $14.25^{a}$ | $8.95^{efg}$ |
| IC582756 | $0.52^{f}$ | $24.20^{fghi}$ | $95.25^{fgh}$ | $30.30^{g}$ | $27.65^{k}$ | $10.22^{c}$ | $8.45^{efg}$ |
| IC582781 | $0.62^{d}$ | $22.70^{hij}$ | $94.37^{ghi}$ | $33.55^{fg}$ | $30.50^{j}$ | $13.05^{b}$ | $11.00^{def}$ |
| IC582783 | $0.46^{g}$ | $28.50^{b}$ | $97.75^{f}$ | $36.35^{efg}$ | $31.00^{j}$ | $14.40^{a}$ | $8.55^{efg}$ |
| IC582788 | $0.36^{i}$ | $25.90^{cdef}$ | $96.50^{fg}$ | $30.90^{fg}$ | $32.35^{i}$ | $15.35^{a}$ | $10.80^{defg}$ |
| IC582800 | $0.32^{jk}$ | $23.30^{ghi}$ | $106.15^{d}$ | $38.25^{def}$ | $32.30^{i}$ | $12.12^{b}$ | $12.20^{cde}$ |
| IC582809 | $0.42^{h}$ | $25.50^{defg}$ | $97.30^{fg}$ | $38.15^{def}$ | $33.20^{hi}$ | $15.42^{a}$ | $9.85^{defg}$ |
| IC582819 | $0.64^{cd}$ | $20.90^{jk}$ | $102.75^{e}$ | $44.95^{bcd}$ | $33.75^{gh}$ | $9.02^{de}$ | $11.70^{def}$ |
| IC582825 | $0.48^{g}$ | $25.60^{defg}$ | $105.60^{de}$ | $55.65^{a}$ | $39.40^{d}$ | $9.55^{cd}$ | $16.20^{abc}$ |
| IC582826 | $0.65^{c}$ | $27.10^{bcde}$ | $109.85^{bc}$ | $44.10^{bcd}$ | $34.65^{g}$ | $12.00^{b}$ | $16.50^{ab}$ |
| PCSAc 1 | $0.42^{h}$ | $27.50^{bcd}$ | $95.50^{fgh}$ | $32.30^{fg}$ | $30.15^{j}$ | $12.20^{b}$ | $7.55^{fg}$ |
| CV (%) | 2.57 | 3.94 | 1.40 | 4.18 | 1.25 | 4.44 | 8.56 |
| CD (0.05) | 0.03 | 2.11 | 2.99 | 3.59 | 0.91 | 1.07 | 2.33 |

**Table 3.** GC MSMS profile of *A. calcarata* volatile oil

| Sl. No. | Compound name | RT | Molecular weight ($g.mol^{-1}$) | Area (%) |
|---|---|---|---|---|
| 1 | à-Pinene | 5.54 | 136.23 | 1.54 |
| 2 | Camphene | 5.79 | 136.24 | 2.03 |
| 3 | Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, | 5.79 | 272.50 | 2.03 |
| 4 | Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)- | 6.23 | 136.23 | 2.75 |
| 5 | Eucalyptol | 7.08 | 154.25 | 19.17 |
| 6 | Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)- | 8.91 | 154.25 | 1.41 |
| 7 | Camphor | 8.91 | 152.23 | 1.41 |
| 8 | à-Terpineol | 9.67 | 154.25 | 1.49 |
| 9 | L-à-Terpineol | 9.67 | 154.25 | 1.49 |
| 10 | Fenchyl acetate | 10.17 | 196.29 | 3.25 |
| 11 | á-copaene | 13.35 | 204.36 | 1.25 |
| 12 | Octadecanoic acid | 22.76 | 284.48 | 1.01 |
| 13 | Eicosanoic acid | 24.61 | 312.53 | 0.60 |
| 14 | Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester | 26.57 | 460.70 | 0.75 |
| 15 | L-Ascorbic acid, 6-octadecanoate | 27.99 | 442.59 | 0.64 |
| 16 | Estra-1,3,5(10)-trien-17á-ol | 28.66 | 334.40 | 1.02 |
| 17 | Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester | 31.88 | 568.90 | 0.90 |
| 18 | 1-Heptatriacotanol | 32.68 | 537.00 | 0.68 |
| 19 | 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis | 33.21 | 444.60 | 0.64 |
| 20 | Glycine, N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris[(trimethyl silyl)oxy] cholan-24-yl]-, methyl ester | 35.46 | 291.61 | 5.04 |
| 21 | 5,8,11-Eicosatriynoic acid, tert-butyldimethylsilyl ester | 36.86 | 414.70 | 6.62 |
| 22 | Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)- | 37.56 | 430.60 | 8.28 |
| 23 | 4aà,4bá-Gibbane-1à,10á-dicarboxylic acid, 4a-formyl-7-hydroxy-1-methyl-8-methylene-, dimethyl ester | 37.90 | 330.41 | 4.28 |
| 24 | Docosanoic acid, 1,2,3-propanetriyl ester | 38.19 | 1059.80 | 1.32 |
| 25 | Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate | 38.34 | 498.78 | 0.65 |
| 26 | 7,8-Epoxylanostan-11-ol, 3-acetoxy | 38.78 | 502.8 | 0.61 |
| 27 | Ethyl iso-allocholate | 39.08 | 436.60 | 15.06 |
| 28 | 1-Monolinoleoylglycerol trimethylsilyl ether | 39.68 | 498.88 | 6.19 |

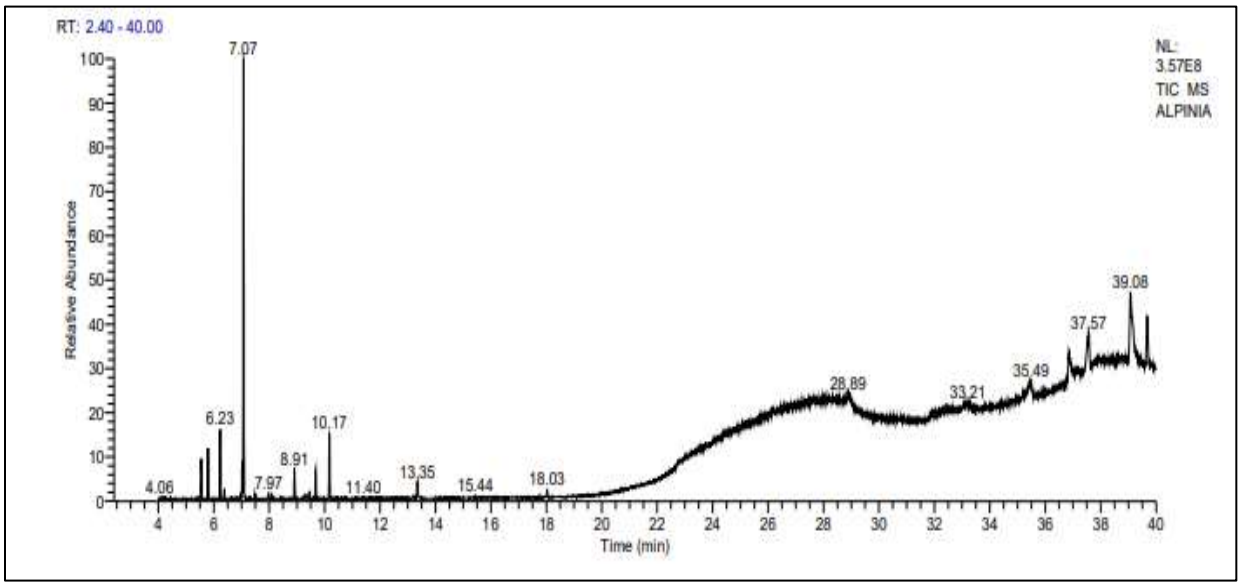


**Fig. 1.** Chromatogram of GC MSMS profile of *A. calcarata* volatile oil

## Conclusion

The rhizomes of *A. calcarata* accessions showed variability in morphological and biochemical characters. Accessions IC373608, IC582825 and IC210421 were found superior with desirable traits and they can be utilized in future crop improvement programmes.

## Acknowledgements

Authors are thankful to Department of Plantation, Spices, Medicinal and Aromatic crops, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur and ICAR NBPGR Regional Station, Thrissur for support and facilities.

## References

Afra N A M & Ghannam H A E 2022 Utilization of rhizome (*Alpinia galanga* L.) in improvement of some quality attributes of some processed meat. Food Nutr. Sci. 13: 761–779.

Arambewela L S, Kumaratunge A, Arawwawela M, Owen N L & Du L 2005 Volatile oils of *Alpinia calcarata* Rosc. grown in Sri Lanka. J. Essent. Oil Res. 17: 124–125.

Arambewela L S R & Arawwawala L D A M 2010 Standardization of *Alpinia calcarata* Roscoe rhizomes. Pharmacogn osy Res. 2: 285–288.

Bhuiyan M N I B, Begum J & Nandi N C 2011 Volatile constituents of essential oils isolated from different parts of *Alpinia calcarata* Rosc. Afr. J. Plant Sci. 5: 349–352.

Datta R, Saxena H O & Kakkar A 2018 Phytochemical evaluation and antibacterial activity of four Indian

species. Asian J. Pharm. Pharmacol. 4: 806–809.

Ferguson N M A 1962 Text Book of Pharmacognosy. Mac Milan Company, New Delhi. pp.191.

Guenther E 1972 The Essential Oils, Vol. 4. Rober E Krieger Publishing Co. Inc., Huntington, New York, pp.150–180.

Hedge J E & Hofreiter B T 1962 In: Carbohydrate Chemistry, 17 (Eds. Whistler RL, Miller JN.), Academic Press, New York.

Indrayan A K, Agrawal P, Rathi A K, Shatru A, Agrawal N K & Tyagi D K 2009 Nutritive value of some indigenous plant rhizomes resembling ginger. Nat. Product Radiance. 8: 507–513.

Jayaweera D M A 1981 Medicinal Plants. Part III, National Science Council of Sri Lanka Publication, Colombo, pp.101.

Jisha M, Hukuman N Z, Leena P & Nair V 2021 Antioxidant, antimicrobial, anticorrosion and molecular docking studies in *Alpinia calcarata* Rosc., rhizome and leaf extracts. J. Mater. Environ. Sci. 12: 244–270.

KAU (Kerala Agricultural University) 2016 Package of Practices Recommendations: Crops (15th Ed.). Kerala Agricultural University, Thrissur, 393p.

Khandel P, Shahi S K, Soni D K, Yadaw R K & Kanwar L 2018 *Alpinia calcarata*: Potential source for the fabrication of bioactive silver nanoparticles. Nano convergence. 5: 1–17.

Mallik C P & Singh M B 1980 Plant enzymology and histoenzymology. A text manual. pp.59–60.

Mathew S, Britto S J & Thomas S 2014 Comparative powder microscopical screening of the rhizome and leaf of *Alpinia calcarata* and *Alpinia galanga*. Int. J. Pharm. Sci. Res. 5: 1449–1453.

Namdeo A G & Kale V M 2015 Comparative pharmacognostic and phytochemical investigation of two *Alpinia* species from Zingiberaceae Family. World J. Pharm. Res. 4: 1417–1432.

Nampoothiri S V, Menon A N, Esakkidurai T & Pitchumani K 2016 Essential oil composition of *Alpinia calcarata* and *Alpinia galanga* rhizomes-a comparative study. J. Essent. Oil Bearing Plants. 19: 82–87.

Nohir G D R, Mostafa U E & El-Hadidy E M 2019 Effect of galangal *(Alpinia galanga*) as hypoglycemic and hypolipidemic agents in diabetic Albino rats. Egypt. J. Nutrition. 3: 337–357.

Ponmozhi, T & Kalaiselvi K 2011 Morphological, histo-anatomical and biochemical studies in *Alpinia* spp. Biochem. Indian J. 5: 109–114.

Raina A P & Abraham Z 2015 Chemical composition of essential oils obtained from plant parts of *Alpinia calcarata* Rosc. (lesser galangal) germplasm from

South India. J. Essent. Oil Res. 27: 238–243.

Raj N, Nadeem S, Jain S, Raj C & Nandi C K 2011 Ameliorative effects *of Alpinia calcarata* in alloxan-induced diabetic rats. Dig. J. Nanomater. Biostructures. 6: 991–997.

Ramya R, Kalaiselvi M, Narmadha R, Gomathi D, Bhuvaneshwari V & Amsaveni R 2015 Secondary metabolite credentials and *in vitro* free radical scavenging activity of *Alpinia calcarata*. J. Acute Med. 5: 33–37.

Sadasivam S & Manickam A 1992 Biochemical Methods for Agricultural Sciences. Wiley eastern limited, 270p.

Sereena K, Kumar U P & Shree A B R 2011 Histochemical and phytochemical marker for the authentication of ayurvedic raw drug hallakam (*Kaempferia rotunda*) and its marketed adulterant. Int. J Pharm. Sci. Res. 2: 2952–2958.

Singh S, Sahoo B C, Kar S K, Sahoo A, Nayak S & Kar B 2020 Chemical constituents analysis of *Alpinia galanga* and *Alpinia calcarata*. Res. J. Pharm. Technol. 13: 4735–4739.

Singh T S, Phucho T & Singh T B 2015 Phytochemical evaluation, determination of total terpenoid content in the rhizome of *Curcuma amada.* World J. Pharm. Res. 4: 2286–2294.

Tewari A, Pant A K, Mathela C S, Mengi N, Kohl E & Bestmann H J 1999 Volatile Constituents of *Alpinia calcarata* Rosc. J. Essent. Oil Res. 11: 739–741.

Trimanto T, Hapsari L & Dwiyanti D 2021 *Alpinia galanga* (L.) Willd: Plant morphological characteristics, histochemical analysis and review on pharmacological properties. In AIP Conference Proceedings (AIP Publishing LLC). 2353: pp.030021.