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RESEARCH ARTICLE

LCMS/MS analysis of methanolic rhizome extracts of *Alpinia calcarata* Roscoe (Zingiberaceae) -A multipotent medicinal plant.

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Abstract

Alpinia calcarata Rosc. of Zingiberaceae popularly known also as Lesser galangal has a widespread occurrence in India, Malaysia, Bangladesh and China. Drugs prepared by using rhizomes of *Alpinia calcarata* are used in the treatment of rheumatism, bronchial catarrah and asthma. They are also used against infection of the skin and also possess antibacterial activity. LCMS/MS study has provided chemical profile present in the methanolic rhizome extract. LCMS/MS full scan was performed on ESI ionization mode using both polarities. Among the 37 compounds identified Catechol, Citral, Protocatechuic acid, Nerol, Umbelliferone, Chrysin, Vanillic acid, Nerolidol have more prominent activities in different levels. The high selectivity of MS-MS detection allowed the development of a very specific and rapid method for the determination of components in methanolic rhizome extracts of *Alpinia calcarata*.

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Introduction:-

Recent years have witnessed a resurgence of interest in traditional medicines and plant derived drugs and return to 'natural cure' all over the world. Natural products are the main sources of bioactive molecules and have played a major role in discovery of lead compounds for the development of drugs for treatment of human diseases (Newman D J *et al* 2007).

Several species of *Alpinia* have been reported to contain several type of flavanoids including chalcones, flavanones, proanthocyanidin, flavonols and flavones (Masuda *et al* 2000), Sesquiterpenes (Miyazawa *et al* 2000), labdane diterpenes (Sy and Brown 1997), Diarylheptanoids (Ali *et al* 2001) and Kava pyrone (Mpalantinos *et al* 1998).

In recent years, Secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju AV *et al* 2005).

Alpinia calcarata is used in the oriental part of the world as a food additive, spice and in indigenous system of medicine. It grows in dense forests at high altitudes and is considered as native of India.

The rhizomes are known to possess a broad spectrum of medicinal properties. is a very good source of pinocembrin 6 which induces mitochondrial apotopsis in colon cancer cells (Kumar *et al* 2007). Experimentally, rhizomes are shown to possess antibacterial (George M and Pandalai KM 1949), antifungal (Pushpangadan P and Atal CK 1984), anthelmintic, antinociceptive (Arambewela LSR *et al* 2004), antioxidant (Arambewela LSR and Arawwawala LDAM 2005), aphrodisiac (Ratnasooriya W D and Jayakody J R 2006), antidiabetic activities (Arambewela LSR *et al* 2009), rheumatism, fever and anticancer activity.

Phytochemical screening revealed the presence of polyphenolic compounds, tannins, flavonoids, steroid glycosides and alkaloids in both Hot Ethanolic Extract (HEE) and Hot Water Extract (HWE) Purushoth prabhu T *et al* (2012).

Liquid chromatography/Mass Spectroscopy (LC/MS) Analysis:-

Liquid chromatography Mass Spectroscopy is an analytical technique for identification, quantitation and mass analysis of a wide variety of non-volatile or semi-volatile organic or inorganic compounds in a mixture. This technique allows for the structural elucidation of unknown molecules through fragmentation. Liquid chromatography coupled with mass spectrometry (LC/MS) is also a powerful technique for the analysis of complex botanical extracts (Cai *et al* 2002).

LC/MS has been widely used to analyse complex mixtures, such as biological samples (Chen Y Z *et al* 2009, Song L J 2005, Spacil Z 2010). An LC-MS is an HPLC system with a mass spec detector. Among hyphenated techniques, LC-MS/MS is the choice of interest because it is highly sophisticated and considerably powerful tool for detection of low and high molecular weight analytes (Jamshed *et al* 2013). In LC/MS, a great advantage of ESI is its ability to provide soft ionization (T Nagata 2006).

Materials and methods:-

Rhizome extracts for phytochemical screening:-

Extraction is the critical step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. The plants were collected from Mannanam, Kerala.

The rhizomes were washed in running water, cut into small pieces and then shade dried for a week at 35-40^{0}C, pulverized in an electric grinder and exhaustively extracted successively in a soxhlet extractor with Petroleum ether, acetone, methanol and water by hot percolation for 3 days. After completion of extraction the dark brown extract was then cooled, filtered, concentrated using rotary evaporator and finally by vaccum suction to get a crude dried extract and yield was calculated, stored in sterile container for further use.

Petroleum ether, acetone, methanol and water extracts were used for the antimicrobial studies. From the results of antimicrobial screening, methanolic extracts showed activity against more bacterial strains. So in present investigation methanolic rhizome extracts of *Alpinia calcarata* were used for LC- MS/MS phytochemical screening.

Phytoconstituents separation by LC- MS/MS:-

LC-MS/MS separate the components according to the molecular weight. LC-MS/MS analysis of crude methanolic extract of rhizome powder were done using LC-MS Shimadzu-SPD 10 AVP apparatus, Japan with a reverse phase C-18.The column used was Phenomenex RP18 with column dimension 25 x 2.5 mm. Mobile phase constitute water: Methanol: THF(50:40:10).10 microliter sample was injected with flow rate of 1.5ml/min with column temperature of 25^{0}C.The ionisation mode of LC-MS/MS was electronic spray ionisation with both positive and negative mode.LC detected at 264nm and the M/Z range was at 50-1000.The software was Class VP integrated and library was METWIN 2.0.

Results and discussion:-

LC-MS/MS Analysis of methanolic extracts of rhizome powder of *Alpinia calcarata:-*

Tandem mass spectroscopy coupled to high performance liquid chromatography (LC-MS/MS), as a sensitive, powerful and robust technique, capable of analysing very diverse complex liquid samples, may offer a solution. LC-MS/MS full scan was performed on ESI ionization mode using both polarities. The positive and negative ESI were obtained shown in fig.1 and fig.2.

Fig.1. LCMS Positive mode Analysis of methanolic rhizome extract of *Alpinia calcarata*

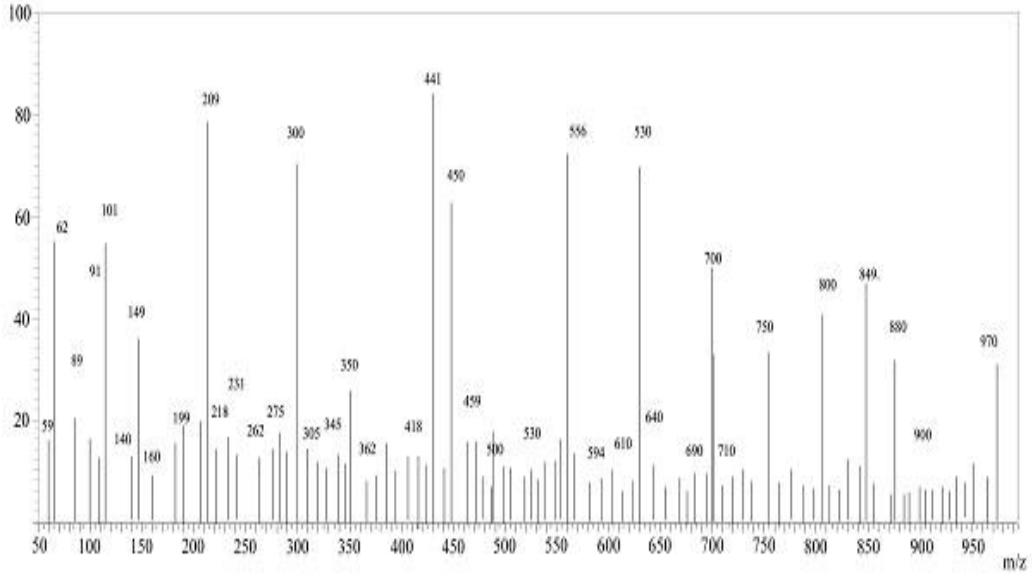
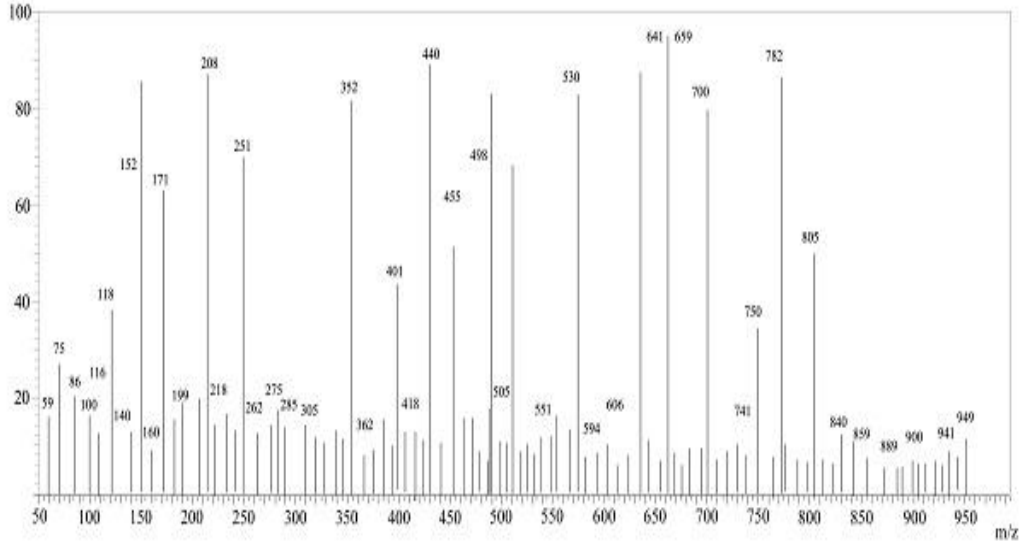


Fig.2. LCMS Negative mode Analysis of methanolic rhizome extract of *Alpinia calcarata*



The high selectivity of MS-MS detection allowed the development of a very specific and rapid method for the determination of components in methanolic rhizome extracts. Electrospray ionization (ESI) was evaluated to get better response of analytes as compared to atmospheric pressure chemical ionization (APCI) mode. The identified compounds are shown in Table -1. The active compounds were checked with the help of Dr. Duke's phytochemical and ethnobotanical databases. Among the identified 37 compounds, Catechol, Citral, Protocatechuic acid, Nerol, Umbelliferone, Chrysin, Vanillic acid, Nerolidol showed more prominent activities indifferent levels (Table-2).

Table. 1 - List of Compounds

Sl. No.	Compound	Molecular Formula	Molecular Mass
1	Beta alanine	$C_3H_7NO_2$	89.10
2	Isovaleric acid	$C_5H_{10}O_2$	102.14
3	Aminobutyric acid	$C_4H_9NO_2$	103.11
4	Catechol	$C_6H_6O_2$	110.11
5	Coumaric acid	$C_9H_80_3$	116.08
6	Diaminobutyric acid	$C_4H_{10}N_2O_2$	118.14
7	Parahydroxy benzaldehyde	$C_7H_6O_2$	122.13
8	Methyl catechol	$C_7H_8O_2$	124.14
9	Thymine	$C_5H_6N_2O_2$	126.12
10	Hydroxy L proline	$C_5H_9NO_3$	131.13
11	4'- hydroxyacetophenone	$C_8H_8O_2$	136.15
12	Alpha terpene	$C_{10}H_{16}$	136.24
13	Para hydroxy benzoic acid	$C_7H_6O_3$	138.13
14	Alpha glutaric acid	$C_5H_6O_5$	146.25
15	Dihydroxyacetophenol	$C_8H_8O_3$	150.14
16	P- Coumaryl alcohol	$C_9H_{10}O_2$	150.18
17	Citral	$C_{10}H_{16}O$	152.24
18	Protocatechuic acid	$C_7H_6O_4$	154.12
19	Isomenthone	$C_{10}H_{18}O$	154.15
20	Nerol	$C_{10}H_{18}O$	154.25
21	Umbelliferone	$C_9H_6O_3$	162.15
22	Coumaric acid	$C_9H_8O_3$	163.16
23	Chrysin	$C_{15}H_{10}O_4$	254.24
24	Deoxykaempferol	$C_{15}H_{10}O_5$	270.25
25	Kaemperol	$C_{15}H_{10}O_6$	286.24
26	Apigenin dimethyl ether	$C_{17}H_{14}O_5$	298.30
27	Vanillic acid	$C_8H_8O_4$	168.15
28	Myristic acid	$C_{14}H_{28}O_2$	228.38
29	Acetoxychavicol acetate	$C_{13}H_{14}O_4$	234.25
30	Nerolidol	$C_{15}H_{26}O$	222.37
31	Himachalol	$C_{15}H_{26}O$	222.39
32	Dihydroresveratrol	$C_{14}H_{14}O_3$	230.37
33	Confertin	$C_{15}H_{20}O_3$	248.33
34	Valerenic acid	$C_{15}H_{22}O_2$	234.34
35	Methyl myristate	$C_{15}H_{30}O_2$	242.40
36	2',6'- Dihydroxy 4' methoxychalcone	$C_{16}H_{14}O_4$	270.29
37	Alpha – linolenic acid	$C_{18}H_{30}O_2$	278.48

Table.2. List of Active Phytochemical compounds

Sl.No	Compound	Antioxidant	Avicide	Neurotoxic	Flavour	Perfumery	Sedative	Allelochemic	Allergenic	Anticancer	Antiseptic	Antibacterial	Antiviral	Antitumour	Antiacne	Cancer preventive	Antiinflammatory	Antispasmodic	Sedative	Antidermatophytoeic	Antiulcer	Anti HIV	Antileukemic	Choleretic	Juvabional	vasodilator
1	Beta alanine	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Isovaleric acid	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Catechol	+	-	-	-	-	-	+	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-
4	Citral	+	-	-	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-	+	-	-	-	-	-
5	Protocatechuic acid	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-	+	+	-	-	-	-	+	-	-	-
6	Isomenthone	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Nerol	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
8	Umbelliferone	-	-	-	-	+	-	+	-	-	+	+	-	-	-	+	+	+	-	-	-	-	-	+	-	-
9	Chrysin	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	+	-	-	+
10	Apigenindi-methyl ether	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
11	Vanillic acid	+	-	-	-	-	-	-	-	+	+	+	-	+	-	+	+	-	-	-	-	-	+	+	-	-
12	Myristic acid	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
13	Acetoxycha-vicol acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
14	Nerolidol	-	-	-	+	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
15	Himachalol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
16	Dihydroresveratrol	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	Confertin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
18	Valerenic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
19	Alpha –linolenic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+

** Source : Dr.Duke's Phytochemical and Ethnobotanical databases
Source : http://ars-grin.gov/duke/

Conclusion:-

Phytochemical analysis conducted on *Alpinia calcarata* rhizome extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the plant studied in the treatment of different ailments. Therefore, extracts from these plants could be seen as a good source for useful drugs.

The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and this plant is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. Therefore, the data generated from these experiments have provided the chemical basis for the wide use of *Alpinia calcarata* as therapeutic agent for treating various ailments.

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