GC-MS Profiling Of Diverse Bioactive Compounds In *Costus Igneus* Leaf Extracts

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Abstract
Costus igneus, popularly recognized as the insulin plant, belongs to the Spiral Flag family *Costaceae* and has been recently introduced in India after originating from South and Central America. Predominantly cultivated as an ornamental plant in southern India, its leaves are also utilized as a dietary supplement in diabetes mellitus treatment. Traditionally acclaimed for its diverse medicinal properties. In this study, comprehensive chromatographic and spectral analyses, particularly using Gas Chromatography-Mass Spectrometry (GC-MS), revealed the presence of various bioactive compounds in *Costus igneus extracts.* Notably, Cyclotrisiloxane, hexamethyl-(cyclic hydrocarbon), a compound with potential therapeutic significance, was identified. Methyl salicylate demonstrated antimicrobial, antioxidant, anti-cancer, and anti-inflammatory properties. Diethyl Phthalate exhibited antimicrobial, acetyl cholinesterase, and neurotoxic activities. The study then focused on the purification and characterization of Cyclotrisiloxane, hexamethyl-. Fraction-1 obtained from the crude ethanolic leaf extract was analyzed using GC-MS and H1, C13 NMR, confirming the presence and providing structural confirmation of the targeted compound.

Keywords: *Costus igneus*, Gas chromatography-Mass spectroscopy (GC-MS), Cyclotrisiloxane, hexamethyl-, Nuclear magnetic resonance (NMR).

Introduction:

Costus igneus, commonly referred as Fiery Costus, Step Ladder, Spiral Flag, or the Insulin Plant, originates from South and Central America and has recently been introduced to India, particularly sought after for its potential as an herbal remedy for diabetes, earning it the colloquial name "Insulin plant" (Jose B, Reddy LJ., 2010). Its presence in Indian gardens as an ornamental plant, thriving both in cultivation and in the wild, underscores its adaptability and widespread acceptance (Benny M., 2004). This versatile plant has a rich history steeped in traditional medicine, with reported medicinal properties spanning anti-diabetic, anti-oxidant, anti-inflammatory, anti-proliferative, anti-urolithiasis, hypolipidemic, neuroprotective, and anti-microbial activities.

Exploration into the secondary metabolites of *Costus igneus* reveals the presence of compounds such as b-sitosterol, corosolic acid, diosgenin, quercetin, catechin, and oleic acid, prominently contributing to its anti-diabetic effects. Recent studies, including those by Shinde *et al*. in 2022, delve into the distinctive morphological, anatomical, and proximate features of *Costus igneus*, offering valuable insights that distinguish it within the broader *Costaceae* family.

In India, the utilization of *Costus igneus* as a means to manage diabetes is noteworthy, with individuals incorporating a daily consumption ritual of one leaf to regulate blood glucose levels (Devi VD, Urooj A., 2008). Further emphasizing its significance, tribal communities in Namakkal district, Tamilnadu, have long recognized the effectiveness of *C. igneus* leaves in diabetes treatment (Elavarasi S, Saravanan K., 2012). This introduction aims to highlight the diverse facets of *Costus igneus*, encompassing its cultural, medicinal, and scientific dimensions.

Materials and Methods:

Sample Collection:

The study involved the collection of plants, *Costus igneus* from the vicinity of Pechiparai and Citraru dams in Kanniyakumari district. After collection, the plants were air-dried for 7 days, followed by the grinding of leaf samples. Gas Chromatography-Mass Spectrophotometer (GC-MS) analysis was performed on the crude ethanolic extract using Agilent Technologies: GC-MS (GC System- 7820A), with specific analysis parameters, including an Over Temp range of -100 ℃to 270 ℃(10 ℃/min), a flow rate of 1.2, and helium gas as the mobile phase.

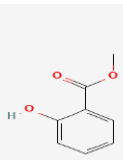
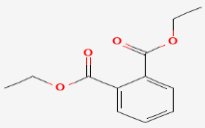
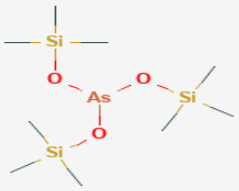
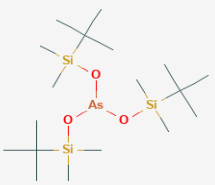
Isolation and Identification of Bioactive Compound:
For the isolation and identification of bioactive compounds, the ethanolic extract underwent compound purification via silica gel column chromatography, employing a mobile phase consisting of ethyl acetate and hexane (1:1). The loading of the ethanolic extract onto the column, elution with solvents, and subsequent analysis of the collected fractions, divided into 6 parts, were conducted. The presence of chromatographic bands in Thin Layer Chromatography (TLC) was assessed by calculating the Rf value.

The focus was particularly on Chromatographic Fraction-1, which was scrutinized for the presence of Cyclotrisiloxane, a hexamethyl-(cyclic hydrocarbon) compound. Subsequently, the structural confirmation of the compound was carried out through Carbon-13 (C13) and Proton (H1) Nuclear Magnetic Resonance (NMR) spectral analysis using the Bruker Avance 400 MHz FT-NMR Spectrometer.

Results:
Identification of Phytocompounds using GC-MS
The identification of phytocompounds in the crude leaf ethanolic extract of *Costus igneus* was carried out using GC-MS analysis. The analysis unveiled the presence of several compounds, including Methyl salicylate (Aromatic carboxylic acid ester) detected at a retention time of 3.619 mins, Diethyl Phthalate (Aromatic carboxylic acid ester) at 7.893 mins, Arsenous acid, tris(trimethylsilyl) ester, Tris(tert- butyldimethylsilyloxy)arsane, and Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl- observed at 17.007 mins. Cyclotrisiloxane, hexamethyl- (Cyclic hydrocarbon) and 3, 3-Diisopropoxy-1, 1, 1, 5, 5, 5-hexamethyltrisiloxane were detected at 17.301 mins. Another compound, Benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-, 4-methyl, exhibited a retention time of 19.664 mins.

Additionally, Thieno [2, 3-c] furan-3-carbonitrile, 2-amino-4, 6-dihydro-4, 4, 6, 6-tetramethyl-, and N-Methyl-1-adamantaneacetamide were identified with peaks observed at 19.712 mins. This comprehensive analysis provides insight into the diverse phytocompounds present in the ethanolic extract of *Costus igneus* leaves, further contributing to our understanding of its chemical composition and potential medicinal properties.(Table: 1., Figure :1)

Table: 1 Identification of compounds in ethanolic extract of *Costus igneus* by GC-MS

S. No	Chemical Compounds	Retent ion Time (min)	CAS Num ber	Molecular Formula	Molecular Structure	Biological Activity
1	Methyl salicylate (Aromatic carboxylic acid ester)	3.619	00011 9-36-8	$C_8H_8O_3$		Anti-Microbial, anti-oxidant and anti-cancer (Essien E. E.,2015) Anti-inflammatory (Li J., 2016)
2	Diethyl Phthalate (Aromatic carboxylic acid ester)	7.893	00008 4-66-2	$C_{12}H_{14}O_4$		antimicrobial, acetylcholinesterase and neurotoxic (Velanganni
3	Arsenous acid, tris (trimethylsilyl) ester	17.007	05542 9-29-3	$C_9H_{27}AsO_3Si_3$		No activity
4	Tris(tert-butyldimethylsilyl oxy)arsane	17.007	10003 66-57-5	$C_{18}H_{45}AsO_3Si_3$		No activity

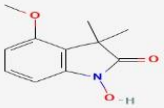
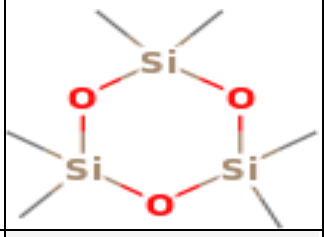
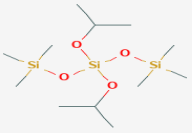
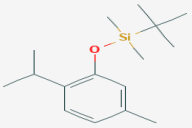
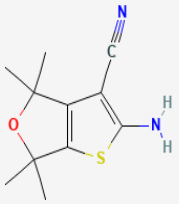
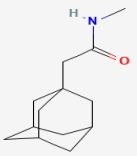
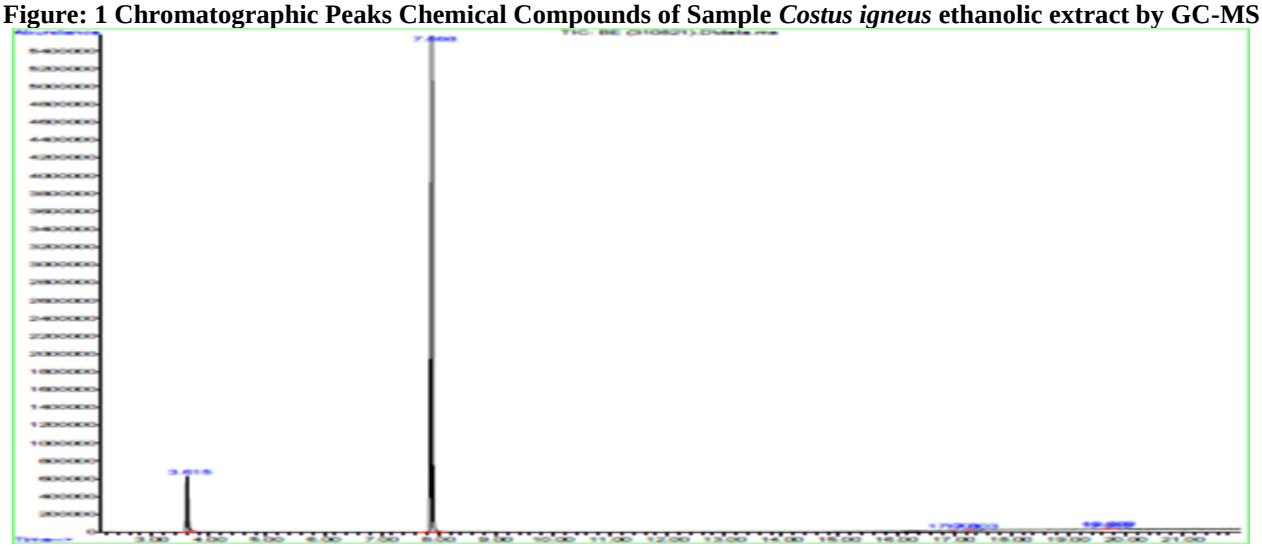
5	Indole-2-one, 2,3-dihydro-N- hydroxy-4-methoxy-3,3- dimethyl-	17.007	10001 29-52-1	C11H13NO 3		Antimicrobial
6	Cyclotrisiloxane, hexamethyl- (Cyclic hydrocarbon)	17.301	00054 1-05-9	C6H18O3S i3		Anti-microbial and Anti-oxidant (S.R, Anjukrishna. *et al*., 2015)
7	3,3-Diisopropoxy-1,1,1,5,5,5-hexamethyltrisilox ane	17.301	01808 2-56-9	C12H32O4 Si3		No activity
8	Benzene, 2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl- 4-methyl-	19.664	33045 5-64-6	C16H28OSi		No activity
9	Thieno[2,3- c]furan-3-carbonitrile, 2-amino-4,6- dihydro-4,4,6,6- tetramethyl-	19.712	44741 2-24-0	C11H14N2 OS		Analgesic, Antianginal, Analgesic, non-opioid, Antihypertensive, Antiarthritic, Dementia treatment, Neurotransmitter uptake inhib itor (Brintha S. *et al*., 2017)
10	N-Methyl-1-adamantaneaceta mide	19.712	03189 7- 93-5	C13H21NO		Antimicrobial (Rateb, H.S. *et al*., 2016) and COX-2 Inhibitor (Kakarla, Lavanya. *et al*., 2014)

Figure: 1 Chromatographic Peaks Chemical Compounds of Sample *Costus igneus* ethanolic extract by GC-MS



Compound isolation and Identification

Spectral Identification of Cyclotrisiloxane, hexamethyl-

Fraction-1, which was subjected to the analysis of selected Cyclotrisiloxane and hexamethyl compounds using the previously employed sample parameters, revealed the presence of Cyclotrisiloxane, hexamethyl, with a retention time of 17.121 minutes. Subsequently, structural characterization of fraction-1 was carried out using H1 and C13 NMR. The H1 NMR spectrum displayed peaks at 18.00 ppm, indicative of the hydrogen atom distribution within fraction-1 (Figure 2). Meanwhile, the C13 NMR spectrum exhibited a peak at 1.42 ppm, corresponding to the carbon skeleton of Cyclotrisiloxane, hexamethyl, and allowed for the prediction of its structure (Figure 3).

Table: 2. Identification Cyclotrisiloxane, hexamethyl Compounds from fraction-1 by GC-MS

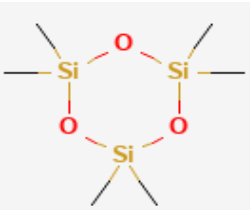
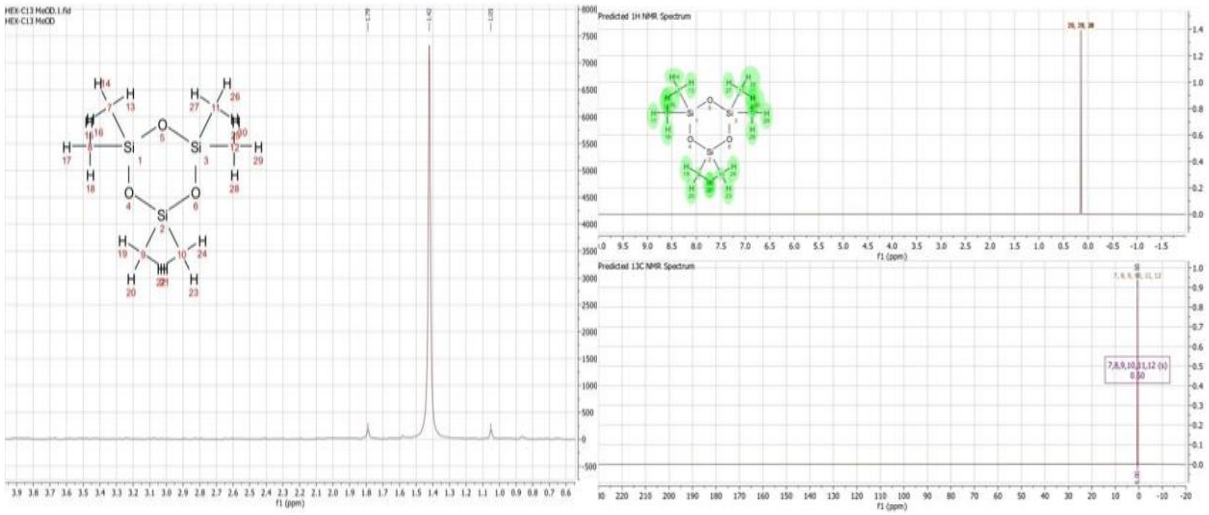
S. No	Chemical Compounds	Retention Time (min)	CAS Number	Molecular Formula	Molecular Structure	Biological Activity
1	Cyclotrisiloxane, hexamethyl-	17.121	000541-05-9	$C_6H_{18}O_3Si_3$		Anti- microbial and Anti-oxidant (S.R, Anjukrishna. *et al*., 2015)

Figure: 3 C13 and H1 NMR spectrum of Cyclotrisiloxane, hexamethyl- from fraction-1 of *Costusigneus*



Discussion

Various plant parts, such as leaves, stems, rhizomes, and roots, contain bio-components. The GC-MS data revealed the presence of bioactive compounds, each with its respective retention time. These compounds were evaluated for their

biological potential, with Methyl salicylate (an aromatic carboxylic acid ester) demonstrating antimicrobial, antioxidant, anti-cancer, and anti-inflammatory properties (Essien, E. E., 2015; Li J., 2016). Diethyl Phthalate (an aromatic carboxylic acid ester) exhibited antimicrobial, acetyl cholinesterase, and neurotoxic activities (Velanganni J., 2011). Cyclotrisiloxane, hexamethyl- (a cyclic hydrocarbon) demonstrated antimicrobial and antioxidant properties (S.R, Anjukrishna *et al*., 2015). Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl- showed analgesic, antianginal, non-opioid, antihypertensive, antiarthritic, dementia treatment, and neurotransmitter uptake inhibitor effects (Brintha S. *et al*., 2017). N-Methyl-1-adamantaneacetamide was reviewed for its antimicrobial and COX-2 inhibitor properties (Rateb, H.S. *et al*., 2016; Kakarla, Lavanya *et al*., 2014).

Among the identified compounds from GC-MS analysis, Cyclotrisiloxane, hexamethyl- (a cyclic hydrocarbon) was selected for purification and characterization. The crude ethanolic leaf extract underwent elution on a silica column and fractionation. Fraction-1 was then characterized for the presence of Cyclotrisiloxane, hexamethyl-, utilizing GC-MS and H1, C13 NMR. The spectral data confirmed the presence and provided structural confirmation of the Cyclotrisiloxane, hexamethyl- compound.

Conclusion:

The GC-MS analysis identified several compounds with diverse biological potentials. Subsequently, the investigation zeroed in on the purification and characterization of Cyclotrisiloxane, hexamethyl-. By subjecting Fraction-1, derived from the crude ethanolic leaf extract, to analysis via GC-MS and H1, C13 NMR, confirmed the presence but also obtained structural confirmation of the targeted compound. The identified compounds, particularly Cyclotrisiloxane, hexamethyl-, exhibited promising biological activities. These findings contribute to the understanding of the therapeutic potential of plant-derived compounds and pave the way for further exploration of their applications in medicine and related fields. Future studies could delve deeper into the mechanisms underlying the observed biological activities and explore potential applications of these compounds in drug development and healthcare**.**

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