Phytoconstituent analysis of *Alpinia galanga* and *Zingiber officinale* plant lipids – A comparative approach

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Abstract:

Phytoconstituents of natural products having a broad spectrum of activity across multiple signaling pathways serve as alternative sources of medicine. Lipids, an essential component of plants, play a crucial role in biological systems for energy storage, as structural components, signaling molecules, modulators of cellular functions and diseases.

Total plant lipids extracted with a biphasic system chloroform - methanol mixed with water facilitated investigation of many compounds including presence of sugars, amino acids, organic acids, waxes, proteins and RNA using TLC. The phytoconstituent properties of the lipids from the medicinal plant *Alpinia galanga, Zingiber officinale* and *Vigna radiata* as comparative standard was further analyzed by FTIR and HPTLC.

The total lipid isolated by Bligh and Dyer method were quantified to be 3.722 g, 7.745 g, 3.429.The percentage yield 14.89%, 30.98%, and 13.716% in *Alpinia galanga, Zingiber officinale and Vigna radiata* respectively. Further studies on sphingolipidomic will define diagnostic or prognostic role of sphingolipids as epigenetic regulators of various human cancers. The lipid fractions of the plant samples isolated and characterized had promising cytoprotective activity on normal cells without causing any side effects and had selective action on cancer cells.

Key words: Phytoconstituent, *Alpinia galanga*, FTIR, HPTLC, Sphingolipids, Glycolipids

Introduction:

Natural products provide a wide variety of phytoconstituents having a broad spectrum of activity across multiple signaling pathways. Natural products disrupt aberrant signaling pathways leading to cancer (i.e., Proliferation, deregulation of apoptosis, angiogenesis, invasion and metastasis) and synergize with chemotherapy and radiotherapy. Researchers constantly turn to natural products for alternative sources of medicine. From the ancient period natural products used for the treatment of various diseases and remarkable numbers of modern drugs have been developed (Kaur et.al, 2011).The plant lipidome is highly complex, lipid composition in different tissues have their specific functions in the development, growth and stress of plants. Efficient lipid extraction protocols will deliver target compounds in solution at adequate concentration for subsequent detection, quantitation and analysis (kehelpannala et al., 2020). Lipids' most essential components of plants play a myriad of crucial roles in biological systems for energy storage, structural components, as signaling molecules and modulators of cellular functions and diseases. Phospholipids and Sphingolipids are major classes of membrane lipids. Sphingomyelins and glycosphingolipids are amphipathic molecules with non-polar aliphatic 'tail' and ceramide with a polar head group. Apoptosis was mediated by sphingolipids and implicated in the mechanism of action in cancer chemotherapeutics (Ogretmen et.al 2004).

In Tumorgenesis oncogenic factor involving activation of antiapoptotic signaling pathways, and proapoptotic tumor suppressor factors, bioactive sphingolipids such as ceramide is associated in regulation of multiple biological functions such as apoptosis (Huang et.al 2004). Endogenous membrane lipids isolated and characterized modulate the viability of tumor cells.

In the present study chloroform, methanol mixed with water, biphasic system was used for extracting total lipids and to investigate many types of compounds including sugars, amino acids, organic acids, waxes, proteins and RNA. Along with lipids from the medicinal plant *Alpinia galanga* to assess anticancer property, *Zingiber officinale* and *Vigna radiata* as comparative standard was further analyzed by TLC, FTIR and HPTLC to identify and characterize the total lipids present in the plants (Sabde et.al, 2011).

Materials and Methods:

Collection of plant samples and Authentication:

Alpinia galangal (L.) Willd (BSI/SRC/5/23/2019/Tech/3217) and *Zingiber officinale Roscoe* (BSI/SRC/5/23/2019/Tech/3218) rhizomes obtained from Sabarimala near the borders of Tamil Nadu and Kerala authenticated by Botanical survey of India – TNAU, Coimbatore were shadow dried, powdered. *Vigna radiata (L.) R. Wilczek* (BSI/SRC/5/23/2019/Tech/3216) seeds were obtained from the department of millets, center for plant breeding and genetics, Tamil Nadu agricultural University, Coimbatore and authenticated by Botanical survey of India – TNAU, Coimbatore.

Total plant lipids extraction:

The total lipids extracted with reference to Bligh and dyer method (1959) and extract containing 100-200 mg lipid evaporated in a water bath at 40-50°C, dry weight of the residue was determined and subtracted from the initial weight.

Weight of the total lipid = (weight of the Petri plate + weight of the extracted plant sample) - (weight of the Petri plate)

Percentage of lipid content (%) = Amount of lipid extracted (g)/ Weight of the plant sample (g)*100

Thin layer chromatography:

The Thin layer chromatography was performed with reference to Robyt and White method, 1987 method, from the lipid fraction, Phospholipids, Phosphatidylcholine (PC), Phosphatidylglycerol (PG), Phospahatidylinositol, Phosphatidylethanolamine (PE), Phosphatidylserine, Sphinolipids, Neutral lipids were identified (Lopez-Rodriguez et al, 2018)

TLC was performed on a Silica gel TLC plate with Chloroform: Methanol: Water (65:16:2) (V/V/V) as the mobile phase. The lipid extract of plant samples prepared with Chloroform: Methanol (2:1) at a concentration of 1mg/ml for the TLC analysis, were developed using Sodium hypochlorite, benzidine reagent, Orcinol reagent, iodine crystals. The retention factor or R_f was calculated.

Fourier Transform Infrared Spectrophotometer Analysis:

Fourier transform infrared spectrophotometer (FTIR) is the most powerful tool to identify types of functional group or chemical bonds present in compounds based on the peak value in the IR radiation region. FTIR spectroscopy is a sensitive and reliable method for the detection of bio molecular composition (Pharmacogn J et al, 2017)

Fourier transform infrared spectroscopy (FTIR) spectra were obtained by means of the Shimadzu model IR Affinity- 1, MIRacle 10 (Serial No: A21374902541). The powered plant samples and lipid extracted plant samples were prepared with Chloroform: Methanol (2:1) at a concentration of 1mg/ml for the FTIR analysis. Spectra were corrected to the base line blank and then plotted.

Quantification of total lipids using High Performance Thin Layer Chromatography:

The HPTLC densitometry analysis was performed to develop a characteristic fingerprint profile, as a marker for evaluation and standardization of drugs . Study reports HPTLC densitometric, a simple, precise, accurate and convenient method for rapid screening of active lipid components for analysis to develop and validate quantification of total lipid extracts from the plant samples. (Kamboj et al.2013).

HPTLC was performed on 10 x 10 Silica gel 60 F 254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument with Hexane / Ethyl ether / Acetic acid (80:20:1 v/v) as mobile phase. The lipid extracted plant samples were prepared with Chloroform: Methanol (2:1) at a concentration of 1mg/ml for the HPTLC analysis. 5 µl of samples and 2 µl of standard solution were loaded as 8mm band lengths . Copper (II) sulfate pentahydrate was dissolved in 200 ml of methanol, sprayed at less than 20° C and under cooling with ice added 8 ml of sulfuric acid 98% and 8 ml of ortho-phosphoric acid 85%. The developed plate was dried by hot air to evaporate solvents from the plate and heat it at 140°C for 30 minutes using a plate heater. The plate was photo documented in visible light, UV 254nm and UV 366 nm mode using CAMAG visualization. After derivatization, the plate was fixed in the scanner stage (CAMAG TLC SCANNER) and scanning was done at UV 366 nm. WINCATS software is used to obtain the peak table, peak display and peak densitogram.

Result and Discussion:

Total plant lipids extraction:

The total lipid content from the plant sample was extracted with reference to the Bligh and dyer method (1959). Table 1 indicates the amount of total and individual lipid constituents present in the plant samples. The total lipids content was 3.722 g, 7.745 g, 3.429 g extracted from 100 – 200 g with percentage yield of 14.89%, 30.98%, and 13.716% in *Alpinia galanga, Zingiber officinale and Vigna radiata* respectively.

Table 1: Quantification of total plant lipid

Serial No	Plant Sample	Amount of total lipids (g)	Percentage of extraction efficiency (%)
1.	*Alpinia galangal (L.) Willd*	3.722	14.89%
2.	*Zingiber officinale Roscoe*	7.745	30.98%
3.	*Vigna radiata (L.) R. Wilczek*	3.429	13.716%

A Soxhlet and re-circulated solvent extraction methods were used to extract lipid materials from grain sorghum in a similar study. The lipid content in sorghum with a moisture content of 6.7% (dry basis) was as high as 9.32% of the dried sorghum as determined by Soxhlet extraction with n-hexane. There was a slight increase in the lipid yield from 9.21% to 9.69% when the moisture contents increased from 2.1% to 17.4% (Huang et al, 2004).

Thin layer chromatography:

The TLC analysis with Benzidine reagent blue spots indicated the presence of Sphingolipids, yellow spots with α-Naphthol solution the Glycolipids and the orange-red spots with Potassium iodide and Bismuth nitrate solution Choline – containing lipids. The complex lipids extracted from plant samples can be separated by thin layer chromatography into different lipids classes. Organic solvent as the mobile phase separates both polar and nonpolar lipids. Depending on the complexity of the lipid samples, lipids can be separated using a 1D or 2D TLC system (Holzl et al, 2021).

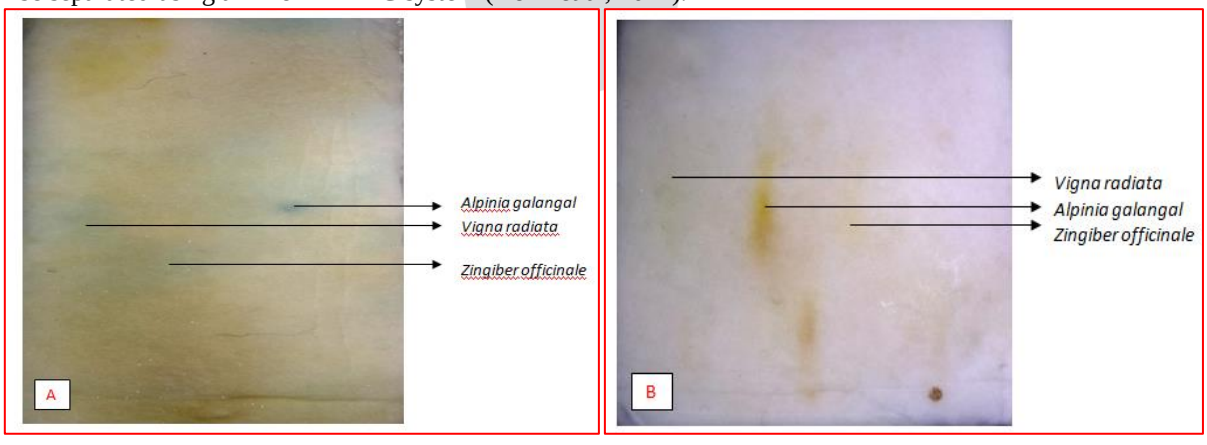


Figure 1: Confirmation test for lipids: A. Detection of Sphingolipids: The TLC plate was developed with Clorox reagent and Benzidine reagent, appearance of blue spots indicates the presence of Sphingolipids in the total lipid extracts of *Alpinia galangal*, *Zingiber officinale* and *Vigna radiata.* **B. Detection of Glycolipids:** The TLC plate was developed with α-Naphthol solution and 95% sulphuric acid solution, appearance of yellow spots indicated the presence of Glycolipids in the total lipids extracts of *Alpinia galangal*, *Zingiber officinale* and *Vigna radiata.*

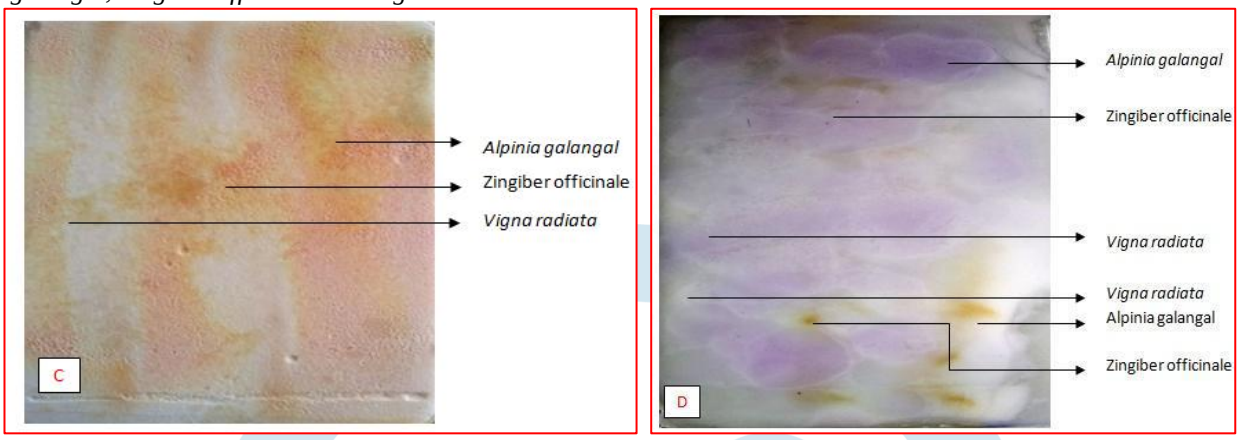


Figure 2: Confirmation test for lipids: C. Detection of choline – containing phospholipids: The TLC plate was developed with potassium iodide and Bismuth nitrate solution, orange-red spots indicates the presence of Choline – containing lipids in the total lipid extracts of *Alpinia galangal*, *Zingiber officinale* and *Vigna radiata.* **D. Detection of lipids with free amino groups:** The TLC plate was developed with Ninhydrin, appearance of red-violet spots indicates the lipids with free amino groups and the TLC plate developed with Resorcinol, violet-blue color and yellow spots indicates the presence of Gangliosides and glycolipids.

Fourier Transform Infrared Spectrophotometer Analysis:

FTIR analysis was carried out to identify the types of chemical bonds (Functional groups) present in the compounds. The peaks were absorbed from 700 to 4000 cm^{-1} in total lipid samples and 400 to 4000 cm^{-1} in powdered plant samples.

FTIR analysis involves the measurement of infrared absorption, relating to a wide range of molecular vibration modes, which is used to identify specific molecular groups and quantification of macromolecules including proteins, lipids, etc. Few studies have demonstrated that FTIR analysis is an effective tool for identifying and monitoring lipid accumulation in microalgae, which is involved in the algal biofuel production process (Dean et.al, 2010).

Graph 1: FTIR analysis - *Alpinia galangal* – Total lipid sample

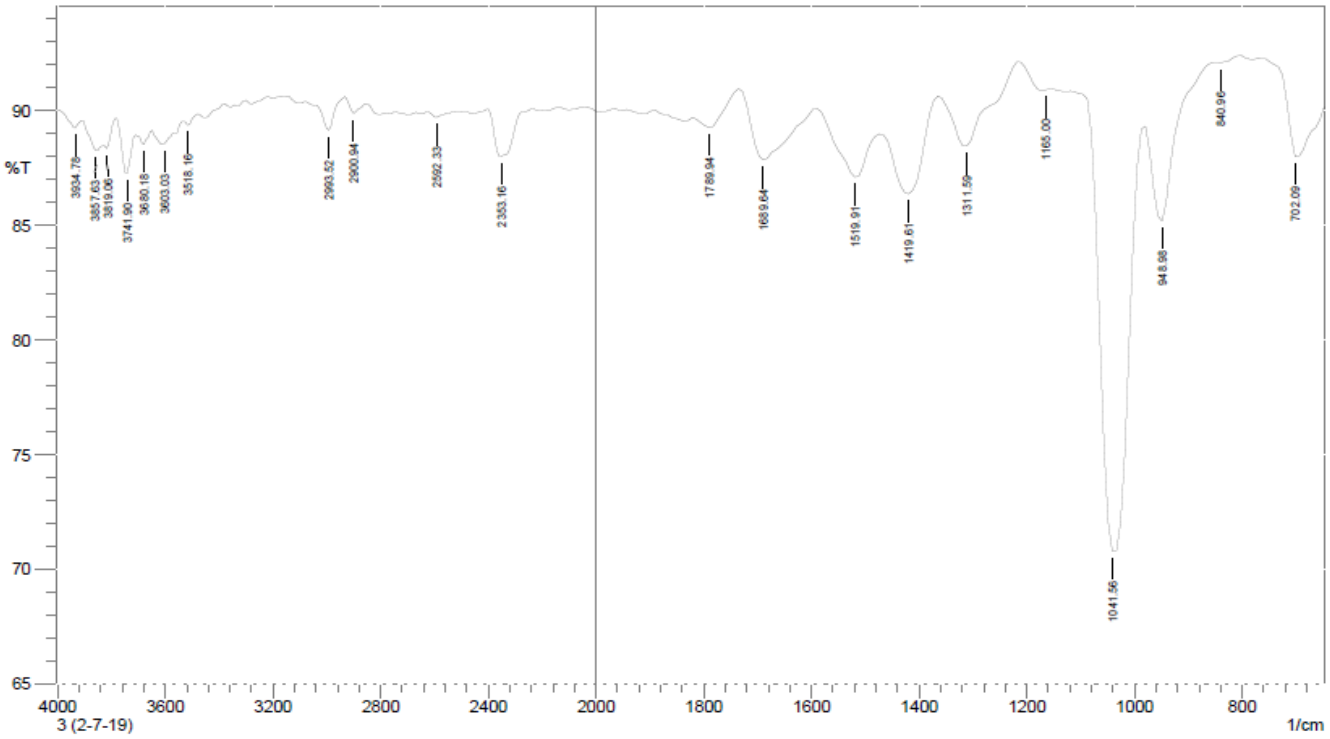


Table 2: FTIR analysis - *Alpinia galangal* – Total lipid sample

Frequency range	Absorption (cm^2)	Appearance	Group	Compound class
4000 - 3000	3741.90	Medium, sharp	O-H stretching	alcohol
	3518.16	Strong, broad	O-H stretching	alcohol
3000 - 2500	2993.52	Medium	C-H stretching	alkane

	2592.33	Weak	S-H stretching	thiol
2400 - 1650	1789.94	Strong	C=O stretching	Conjugated acid halide
	1689.64	Weak	C-H bending	Aromatic compound
1400 - 1000	1519.91	Strong	N-O stretching	Nitro compound
	1419.61	Medium	O-H bending	alcohol
	1311.59	Medium	O-H bending	Phenol
	1041.56	Strong, broad	CO-O-CO Stretching	Anhydride
900 - 700	840.96	Strong	C-CI stretching	Halo compound
	702.09	Strong	C=C bending	alkene

Graph 2: FTIR analysis – *Zingiber officinale* – Total lipid sample

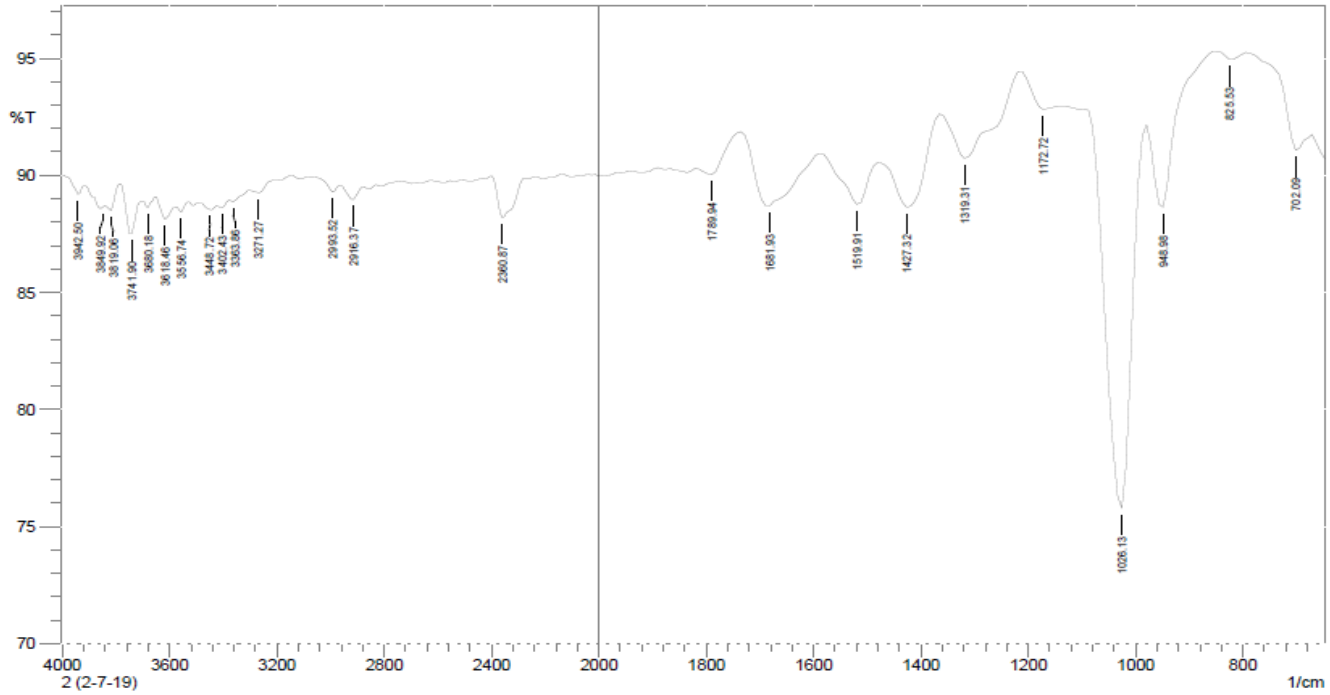


Table 3: FTIR analysis – *Zingiber officinale* – Total lipid sample

Frequency range	Absorption (cm^2)	Appearance	Group	Compound class
4000 - 3000	3741.90	Medium sharp	O-H stretching	alcohol
	3448.72	Strong, broad	O-H stretching	alcohol
3000 - 2500	2993.52	Strong, broad	N-H stretching	Amine salt
2400 -2000	2360.87	strong	O=C=O stretching	Carbon dioxide
	2349	strong	O=C=O stretching	Carbon dioxide
2000 - 1650	1789.94	Strong	C=O stretching	Conjugated acid halide
	1681.93	Strong	C=O stretching	Conjugated acid
1400 - 1000	1519.91	Strong	N-O stretching	Nitro compound
	1427.32	Medium	O-H bending	Carboxylic acid
	1319.31	Strong	S=O stretching	Sulfone
	1026.13	Medium	C-N stretching	amine
900 - 700	825.53	Medium	C=C bending	alkene
	702.09	Strong	C=C bending	alkene

Graph 3: FTIR analysis - *Vigna radiata*– Total lipid sample

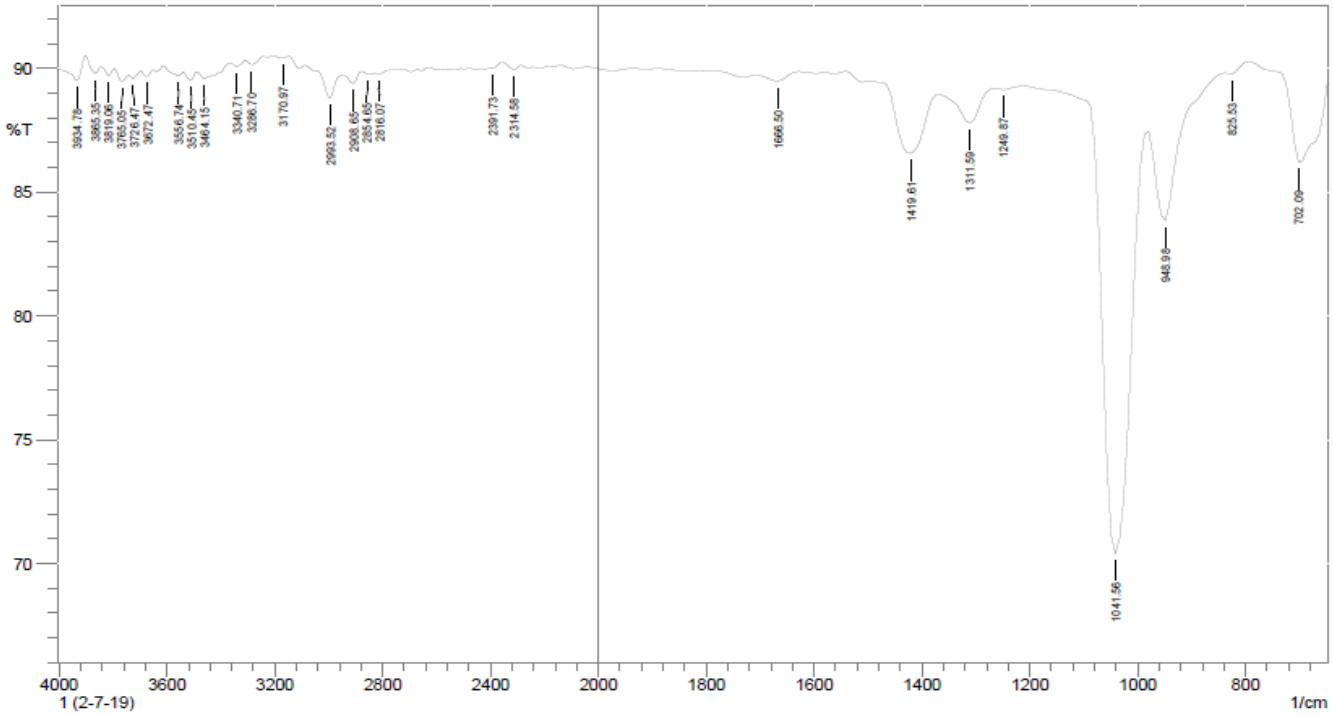


Table 4: FTIR analysis - *Vigna radiata*– Total lipid sample

Frequency range	Absorption (cm^2)	Appearance	Group	Compound class
4000 - 3000	3765.05	Medium Sharp	O-H stretching	alcohol
	3464.15	Strong, broad	O-H stretching	alcohol
	3170.97	Weak, broad	O-H stretching	alcohol
3000 - 2500	2993.52	Medium	C-H stretching	alkane
	2908.65			
	2854.65	Medium	C-H stretching	aldehyde
	2816.07			
2400 - 2000	2391.73	Strong	O=C=O stretching	Carbon dioxide
	2314.58			
2000 - 1650	1666.50	Strong	C=O stretching	Conjugated ketone
1400 - 1000	1311.59	Strong	C-O stretching	Aromatic ester
	1249.87	Medium	C-N stretching	amine
	1041.56	Strong, broad	CO-O-CO Stretching	Anhydride
900 - 700	825.53	strong	C-CI stretching	Halo compound
	702.09	Strong	C=C bending	alkene

Quantification of total lipids using High Performance Thin Layer Chromatography:

HPTLC analysis was carried out to confirm the presence of Lipids. Black, blackish blue colored fluorescent zone at UV 366 nm and visible light mode present in the track as observed from the chromatogram after derivatization confirmed the presence of phospholipids in the samples and standard.

Table 5: HPTLC Analysis - Peak table

Track	Peak	Rf	Height	Area
Alpinia galanga	**1**	**0.55**	**127.4**	**2616.9**
Zingiber offficinale	**1**	**0.51**	**248.6**	**5239.0**
Vigna radiata	**1**	**0.52**	**277.2**	**6349.4**
Standard (Cholesterol)	**1**	**0.53**	**641.7**	**21953.3**

HPTLC – Chromatogram:

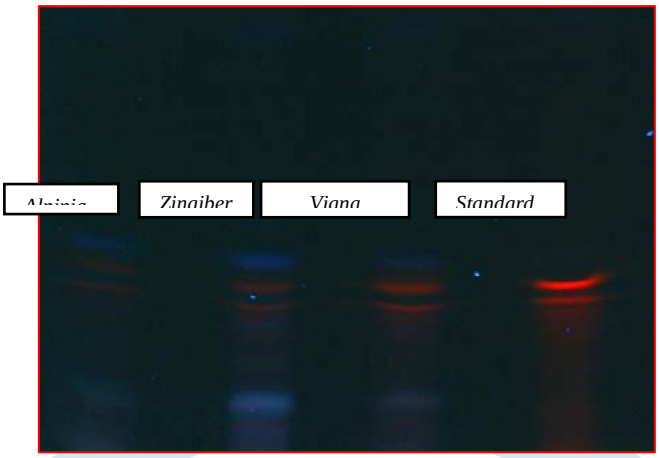
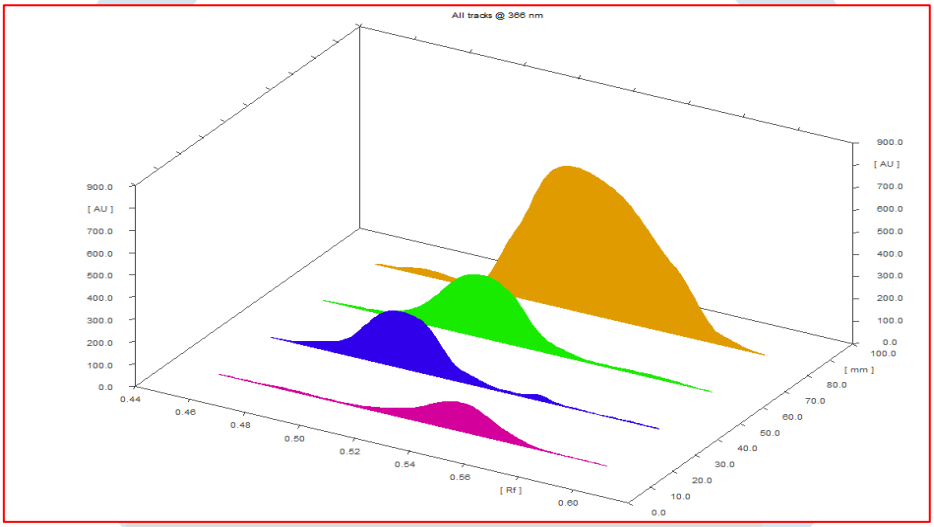


Figure 3: HPTLC chromatoplate of Total lipid extracts of *Alpinia galanga*, *Zingiber officinale*, *Vigna radiata* and standard (Cholesterol) after derivatization under 366 nm.



Graph 4: 3D densitometric chromatogram of Total lipid extracts of *Alpinia galanga*, *Zingiber officinale*, *Vigna radiata* and standard (Cholesterol) after derivatization under 366 nm.

Summary and Conclusion

Alpinia galanga (Greater galangal) is a species of ginger which occurs in the areas of Tropical Asia and was used as a traditional medicine and condiment in many countries. The phytochemical constituents of rhizomes have anti-tumor, anti-ulcer and anti-fungal properties. The essential oils and extracts of greater galangal rhizomes have been studied and significantly inhibit the growth of tumor cells (Subramanian et.al, 2015).

This study aimed to determine analytical methods for extraction of lipids from plant samples *Alpinia galanga*, *Zingiber officinale* and *Vigna radiata* using Chloroform: methanol (2:1) solvent by Bligh and Dyer method and the total lipid content were quantified to be 3.722 g, 7.745 g, 3.429 g extracted from 100 – 200 g with percentage yield of 14.89%, 30.98%, and 13.716% in *Alpinia galanga, Zingiber officinale and Vigna radiata* respectively.

The TLC and HPTLC analysis to detect the presence of lipids like Sphingolipids, Glycolipids and Phospholipids when developed with Clorox reagent and Benzidine reagent blue spots indicated the presence of Sphingolipids, with α-Naphthol solution and 95% sulphuric acid solution yellow spots indicated the presence of Glycolipids. Potassium iodide and Bismuth nitrate solution, orange-red spots the presence of Choline – containing lipids, Red-violet spots with Ninhydrin indicated lipids with free amino groups. Violet-blue color and yellow spots with Resorcinol indicated the presence of Gangliosides and glycolipids.

FTIR and GCMS confirmed the function groups by relating the intensities to those of the lipid content at specific wavelengths. Targeted lipid profiling a comprehensive approach provides intriguing new insights into sample contents while considering several factors for selecting optimal methods for extraction of total plant lipids. Easy and rapidity, reproducibility of the method, cost-effectiveness, routine lipidomic analysis, sample recovery, effective removal of interference in large scale are other important factors to be considered.Further studies on sphingolipid comic will define diagnostic or prognostic role of sphingolipids as epigenetic regulators of various human cancers. The lipid fractions of the plant samples isolated and characterized had promising cytoprotective activity on normal cells without causing any side effects and had selective action on cancer cells. Cancer therapy resistance is major problems leading to treatment failure mounting evidence indicate the apoptotic sphingolipid- ceramide as important suppressor in cancer development. Alterations of ceramide metabolism become a strategy for cancer cells to develop resistance against therapy. Conversely, manipulation of ceramide metabolism provides potential alternative and combinational therapeutic options. By accumulating knowledge about how cancer cells escape from apoptotic stimuli and the discovery of potent and safe inhibitors, targeting ceramide metabolic pathways provide opportunities for more feasible and efficacious cancer

therapy.Sphingolipids recognized as a diverse and dynamic regulator of cellular processes though viewed as primarily inert structural compounds of cellular membrane, due major development has led to the realization of sphingoid bases, ceramides and intermediates of sphingolipid metabolism act as a signaling molecules in mediating cell cycle control, stress responses and apoptosis (Ryan et.al, 2004).Sphingolipid contributes to the stability of other types of biological structure as the lamellar bodies that maintain the permeability barrier of skin and lipoproteins, which also have messenger functions that regulate the proliferation, survival and death of cells. Cytotoxic and exposure of cells to radiation or chemotherapy damages can be prevented by exogenous application of ceramide and is associated with increased ceramide levels due to enhanced de novo synthesis, catabolism of sphingomyelin (Reynolds CP et.al, 2004)

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