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Research Article

Phytochemical Screening, GC-MS and FTIR analysis of Bioactive Compounds in Methanolic Leaf Extracts of *Costus igneus* (CIMLE) as a Natural Source of Drug Lead Molecules for Next-generation Drug-design, Development and Therapeutics

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Abstract

Diabetes mellitus (DM) is a metabolic disorder that affects people of all ages. Increased prevalence of diabetes worldwide has led to the development of synthetic drugs to provide an interim solution to the ill effects of the issue. However, such drugs, although effective as antihyperglycemic agents, are accompanied by significant side effects, costly, and inaccessible to majority of people in remote in underdeveloped countries. Local medicinal plants have been used by such indigenous people through ages to treat such complicate ailments due to their ease of availability and GRAS nature. As diabetes continues to become prevalent, health care practitioners are considering PBNPs as a potential source of antidiabetic drugs due to their high potency and fewer side effects. To better understand the mechanism of action of medicinal plants, their active phytoconstituents are being isolated and investigated thoroughly. Phytochemical screening followed by GCMS analysis of methanolic leaf extracts of *Costus igneus* depicted the presence of 19 bioactive compounds viz, Bicyclo [3.1.1]heptane, 2,6,6- trimethyl-; 2-Pentadecanone, 6,10,14-trimethyl-; phthalic acid; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; 5,9,13-Pentadecatrien-2-one, 6,10,14- trimethyl; 2(3H)-Furanone, dihydro-5-methyl-5- pentyl; Hexamethyl-2,6,10,14,18,22- tetracosahexaen-1-yl]-6-methox.; Oxalic acid, cyclohexyl isohexyl ester; 6-Octadecenoic acid; Phytol; 4,8,12,16-Tetramethylheptadecan-4- olide; 5-methyl-2,3-dihydro-1H-indole; Bis(2-ethylhexyl) phthalate; Adamantane-1-carboxylic acid methyl ester; Benzopyran-6-ol,3,4-dihydro-; Benzoquinoline; gamma-Tocopherol; dimethyl-5,5'-diphenyl-1H; 1H-benzo[b]furo[2,3-f]indole that may be ADMET predicted for potential biomedical application in the treatment of diabetics and long term complications associated with the disease.

Keywords: *Costus igneus*; CIMLE; GCMS; Phytochemical Screening; Antidiabetic Leads; NDDT; Antidiabetic Medicinal Plant

INTRODUCTION

According to World Health Organization (WHO), 80% of the global population uses medicinal-plant-based medicine to alleviate or cure diseases.¹ In addition, although estimates vary depending on what is considered a natural-product-derived drug, it is safe to say that up to 50% of currently marketed drugs owe their origins to natural products.² New molecules from natural resources with potential bioactivity are reported every day; however, only a few of these molecules are evaluated for their suitability for use as drugs.³ Identifying bioactive compounds (hits or leads) is the initial step for drug discovery. Therefore, it is necessary to select suitable bioassays to evaluate both the activity against the disease and the potency. For this purpose, target-based screening is mainly used to identify compounds that modulate the activity of a

target involved in a disease. This screening involves different in vitro biological assays designed to measure primary activities, selectivity, cellular toxicity, and physiologically relevant activity. The initial phases of a target-based screening cascade typically employ a range of in vitro assays, especially high-throughput screening (HTS); however, the study is more expensive and time-consuming.⁴ In the first instance, the assays can be selected considering that structurally similar compounds have similar biological activity; however, this cannot always be carried out, especially when working with natural compounds or natural extracts.

Extracts from various plants are commonly used in traditional medicine, either alone or in combination, but in many cases only a few of them are evaluated for their biological activity. However, due to inadequate fractionation processes or the

degradation of active compounds during separation, it is not always easy to identify the molecules responsible for the activity of these extracts. Since obtaining an isolated compound very often requires infrastructure and specialized personnel and is expensive, it is challenging to offer pharmacological alternatives of this nature to people with low incomes. In addition, since medicinal herbs can be grown locally at a reasonable cost, natural extracts remain an option for treating some diseases in populations with limited resources or living in remote areas.⁵

An important advantage of using crude extracts vs. isolated molecules is the presence of molecules in the extract that can interact synergistically with the bioactive compound, potentiating its beneficial effect.⁶ However, despite their potential as accessible treatment options and as sources of bioactive molecules for drug discovery, it must be highlighted that similar to other pharmacological alternatives, natural extracts can also present adverse effects that should be considered and evaluated. Having many assays on hand to discard or confirm activities is critical when looking for bioactive compounds because the aim while looking for therapeutic agents is to identify an acceptable technique that can screen the source material for bioactivity.

Currently, there are numerous antidiabetic agents available for the treatment of diabetes mellitus (DM), which target different receptors.⁷ The most important classes of antidiabetic oral medicines include biguanides, such as metformin, sulfonylureas, meglitinide, thiazolidinedione, dipeptidyl peptidase 4 inhibitors, sodium glucose cotransporter (SGLT2) inhibitors and α -glucosidase inhibitors.⁸ Sulfonylureas increase insulin secretion by blocking KATP channels and therefore lower blood glucose levels. They are divided into first-generation agents, such as tolbutamide, chlorpropamide, acetohexamide, metahexamide and tolazamide, and second-generation agents, such as glipizide, glyburide, gliclazide, glibenclamide and glimepiride, which are sometimes also considered third-generation agents.⁹ Another class of drugs for the treatment of DM is meglitinides (glinides), which include repaglinide and nateglinide. Diazoxide (DZ) is a direct insulin secretion inhibitor that is often used for the treatment of insulinoma, a rare neuroendocrine tumor of the pancreas that leads to hypoglycemia. DZ inhibits insulin release by opening KATP channels, in contrast to sulfonylureas, which stimulate insulin secretion by blocking KATP channels.¹⁰

Medicinal plants are used extensively as drugs for various diseases. Especially in developing countries, medicinal plants are used to treat DM due to the costs of conservative medicines.¹¹ Medicinal plants are a source of biological and chemical compounds that are important pharmaceuticals and are currently an important tool for the identification of novel drug lead compounds. A large number of plants, their extracts and their phytochemicals have been shown to affect the insulin secretion mechanism.¹² *Galega officinalis*, a plant that contains biguanide, has been used since the middle ages for the treatment of diabetes.¹³ Several plant species are known for their antidiabetic properties, and a variety of plant extracts have been described to have valuable antidiabetic treatment effects. Importantly, these plants and their extracts are considered to be less toxic and have fewer side effects than synthetic drugs.¹⁴⁻¹⁶ On the one hand, plant extracts can be used as complementary and alternative remedies to prevent metabolic diseases, and on the other hand, they are an interesting source of compounds for potential new drug candidates.¹⁷

Costus igneus Nak (syn. *Costus pictus* D. Don, *Costus mexicanus* Liebm ex Petersen or *Costus congenitus* Rowle), commonly known as fiery *Costus*, Step ladder or Spiral flag or Insulin plant, is native to South and Central America. This plant has been introduced to India from America as an herbal cure for diabetes and is popular as 'insulin plant'.¹⁸ CI is a perennial, upright, herb grows up to 2 ft, in group from the rhizome. Leaves simple, alternate, entire, oblong, evergreen, 4- 21 inches in Lt with parallel venation (Prakash et al., 2014). Leaves - large, smooth, underside of leaves is shiny and spirally arranged around stems, aerial shoot arise from underground rootstocks (Prakash et al., 2014, Nayagam, 2015). Yellow flowers with red stripes (1.5-inch dia), appear on cone-like heads at tip of branches, and usually bloom during summer. CI is propagated by aerial stem cutting, adventitious buds and rhizome. *Costus* species has been used in folk medicine. CI contains diosgenin, steroid saponins (prosapogenin, α & β dioscin), furostanol saponins (costunolide, octasanoic acid, cycloartenol). The plant exhibits hypolipidemic, diuretic, ameliorative, antimicrobial (antibacterial and antifungal), anticancer and putative agent of antidiabetic, hepatoprotective, antifertility, and antioxidants such as vitamin C, β -carotene and phenolic compounds (Vidigal Minim et al., 2011) activity. Trace elements like nitrogen, calcium, potassium, sodium, magnesium and phosphorus (Singh (2011) Ukana et al., (2012). screening for phytochemicals of *C. igneus* leaves revealed that it is rich in protein, iron, and antioxidant components such as ascorbic acid, α -tocopherol, β -carotene, terpinoids, steroids, and flavonoids.¹⁹⁻²¹

MATERIALS AND METHODS

Phytochemical Screening

C. igneus plant was collected from Botanical Garden maintained by Department of Botany, E.M.G. Yadava Women's College (A) Madurai, TamilNadu, India. Identity of the plant specimen was established followed by authentication at PG Department of Government Arts College, Melur, Madurai India. The plant material was cleaned in distilled water (D.H₂O) containing 2% mercury chloride followed by rinsing with double distilled water. Subsequently, the plant material was shade-dried at RT (37°C). Leaves were cut and pulverised to fine powder at RT. The fine powder was processed for the preparation of extract in 70% methanol (solvent) in an accelerated solvent extractor at -20 °C and 15 atm pressure. The standard operational method was used to ensure quality during the preparation of extract (Fibigr et al., 2018). The extract was allowed to dry at optimum temperature (37°C). The yield of *C. igneus* was 70%. The dried extract was stored in a refrigerator at 4°C till further screening.

Phytochemical Screening/ GCMS, FTIR Analysis

The methanolic leaf extracts of *Costus igneus* were subjected to various chemical tests for the detection of phytoconstituents and GCMS, FTIR analysis using standard procedures described previously²²⁻⁴⁰.

TEST FOR ALKALOIDS

Mayer's test: Few drops of Mayer's reagent was added to 1 mL of plant extract, appearance of a deep yellow or white precipitate indicated the presence of alkaloids in the solution. (Mayer's reagent was freshly prepared by dissolving mercuric chloride (1.36 g) and potassium iodide (5.00 g) in 100 ml water).

Dragendorff's test: To 2 mL of the extract added 1 mL of Dragendorff's reagent along the side of the test tube. Formation of orange or orange reddish brown precipitate

indicated the presence of alkaloids. Dragendorff's reagent was prepared by Sol A: 0.85g bismuth subnitrate, 40mL water, and 10mL glacial acetic acid and Sol B: 8g potassium iodide and 20mL water. 5mL each of Sol A & B with 20mL of glacial acetic acid and 70-100 mL of water is mixed to prepare Dragendorff's reagent.

Hager's test: Hager's test was done by adding a few drops of Hager's reagent to plant extracts and appearance of a yellow-color precipitate indicated the presence of alkaloids in the solution. Hager's reagent is saturated solution of picric acid.

Wagner's test: Approximately, 1 ml of crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown colour precipitate indicates the presence of alkaloids. Wagner's Reagent was prepared by mixing 2.5 gm iodine in 12.5 gm of potassium iodide (KI 2); add 250 ml of water to produce solution.

TEST FOR GLYCOSIDES

Test for Anthraquinones Glycosides

Bornträgers test: 0.5 g of extract was boiled with 10% hydrochloric acid for few minutes in water bath. It was filtered and allowed to cool. Equal volume of CHCl_3 was added to the filtrate. Few drops of 10% ammonia was added to the mixture and heated. Formation of rose - pink color indicates of n-hexane, chloroform, ethyl acetate and methanol of the presence of the anthroquinones.

Baljet test: Part of plant containing cardiac glycoside is dipped in sodium picrate solution; formation of a yellow to orange colour indicates the presence of aglycones or glycosides in the plant tissues.

Legal's Test: To the concentrated ethanolic extract few drops of 10% NaOH were added, to make it alkaline. Then freshly prepared sodium nitroprusside was added to the solution. Presence of blue coloration indicated the presence of glycosides in the extract.

TEST FOR CARDIAC GLYCOSIDES

Keller-Kiliani test: 5 ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1 ml of concentrated sulphuric acid. A browning of the interface indicates a deoxy-sugar characteristic of carotenoids. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

TEST FOR CARBOHYDRATES

Molisch's test: Small portion of the plant extract was put in a test tube; 10 ml of distilled water was added and shaken vigorously and gently. The mixture was then filters and divided into two portions. To the first portion, two drops of Molisch's reagent was added followed by few drops of concentrated sulphuric acid by the wall of the test tube. Formation of brown or purple ring at the interphase indicated the presence of carbohydrates.

Fehling's test Equal volume of Fehling A and Fehling B reagents were mixed together and then add 2ml of crude extract in it and gently boiled. A brick red precipitate appeared at the bottom of the test-tube indicates the presence of reducing sugars.

Benedict's test 1 ml of crude extract was mixed with 2ml of Benedict's reagent and boiled. A reddish brown precipitate was formed which indicates the presence of the carbohydrates.

TEST FOR PHYTOSTEROLS

Liebermann Burchard's Test: Dissolve one or two crystals of cholesterol in dry chloroform in a dry test tube. Add few drops of acetic anhydride and then 2 drops of concentrated H_2SO_4 and mix well. The formation of a green or green-blue colour after a few minutes indicates the presence of phytosterols. After the reaction, concentration of cholesterol can be measured spectrophotometry.

Salkowski's Test: On adding a few drops of conc. Sulphuric acid to the plant extract and allow the solution to stand for some time, formation of brown ring indicated the presence of phytosterols in the plant extract.

TEST FOR FLAVONOIDS

FeCl₃ Test: To 1 ml of the extract, 3 ml of distilled water followed by few drops of 10% aqueous Ferric chloride solution was added. Formation of blue or green colour indicates the presence of flavonoids. **Shinoda Test:** To 2 ml of the extract, 1 ml of 1% ammonia solution was added. Appearance of yellow colour indicates the presence of flavonoids.

Shinod's Test: In this test, four pieces of magnesium fillings (ribbon) are added to the ethanolic extract followed by a few drops of concentrated hydrochloric acid. A reddish colour indicates the presence of flavonoid.

TEST FOR FIXED OILS AND FATS

Spot test: Take the sample to be tested, press a little in the folds of the filter paper. On folding, if there is the appearance of greasy spot indicates the presence of oils or fats. The spot grows larger on heating and drying the filter paper.

Saponification: Take approximately 100 mg of oil or fat in a test tube. Add 3 mL of alcoholic-KOH and mix well. Place the tube in a boiling water bath for 15-20 min. Saponification value represents mg of potassium hydroxide required to saponify one gram of fat under the conditions specified. It is a measure of the average molecular weight of all the fatty acids present in the sample as triglycerides.

TEST FOR FREE AMINO ACIDS

Millon's reagent test: Millon's test is specific to phenol containing structures (tyrosine is the only common phenolic amino acid). Millon's reagent is concentrated HNO_3 , in which mercury is dissolved. As a result of the reaction a red precipitate or a red solution is considered as positive test.

Ninhydrin reagent test: A 2% solution of ninhydrin is prepared by dissolving 0.2 grams of ninhydrin in 10ml of either ethanol or acetone. 1% solution of the amino acid (analyte) in distilled water is prepared, few drops of 2% ninhydrin solution is added to this solution. Test tube is kept in a warm water bath for 5 min; development of a deep blue/violet colour indicates presence of amino acids.

TESTS FOR FIXED OILS AND FATS

Spot test Take the sample and place it between the folds of filter paper and rub it lightly. Presence of translucent spots on the filter paper confirms the presence of fats in the plant material.

Saponification Take a sample a test tube, add strong alkali NaOH, boil the solution in a water bath for 5 min, add ethanol. Observe for the appearance of froth, formation of forth in the test tube indicates the presence of fat in the sample.

TEST FOR FREE AMINO ACID

Millon's test 1 ml of crude extract was mixed with 2ml of Millon's reagent; white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Ninhydrin test 1 ml of crude extract was mixed with 2ml of 0.2% solution of Ninhydrin and boiled. A violet colour precipitate was appeared suggesting the presence of amino acids and proteins.

TEST FOR TANNINS

5% Ferric chloride test: 5 mg of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black color indicates the presence of tannins.

10% Lead acetate test: 10 mg of extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

TEST FOR SAPONINS

Foam Test: 2 ml of crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins.

GUMS & MUCILAGE

Ruthenium red test: 50 mg of dried mucilage powder was dissolved in 2 mL of distilled water, mixed with a few drops of Ruthenium red solution. Observed for pink color indicates the presence of gums and mucilage.

GC-MS Analysis:

Leaf samples of were collected from University Campus, Vellore, Tamilnadu, India. Phyto-components were identified using GC-MS detection system as described previously, however with modification, whereby portion of the extract was analyzed directly by headspace sampling. GCMS analysis was accomplished using an Agilent 7890A GC system set up with 5975C VL MSD (Agilent Technologies, CA, and USA). Capillary column used was DB-5MS (30 m × 0.25 mm, film thickness of 0.25 µm; J&W Scientific, CA, USA). Temperature program was set as: initial temperature 50°C held for 1 min, 5°C per min to 100°C, 9°C per min to 200°C held for 7.89 min, and the total run time was 30 min. The flow rate of helium as a carrier gas was 0.811851 mL/ min. MS system was performed in electron ionization (EI) mode with Selected Ion Monitoring (SIM). The ion source temperature and quadrupole temperature were set at 230°C and 150°C, respectively. Identification of phyto-components was performed and confirmed by comparison of their retention times and mass with those of authentic standards spectra using computer searches in NIST 08.L and Wiley 7n.l libraries.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The methanolic leaf extracts of CI (CIMLE) were subjected to FTIR analysis as describe previously, using Agilent Cary 630 FTIR spectrometer equipped with Micro-lab PC software with ATR sampling unit with a resolution of 8 cm⁻¹ and scan range of 4000 cm⁻¹ to 650 cm⁻¹.

RESULTS AND DISCUSSION

Phytochemical screening of bioactive compounds in the methanolic leaf extracts of *Costus igneus* (CIMLE) Test for Alkaloids in the CIMLE using Mayer's Test (+++); Dragendorff's Test (+++); Hager's Test (+++); Wagner's Test (+++) showed the presence of alkaloids⁴¹; Test For Anthroquinone Glycosides in the leaf extracts of CI using

Borntrager's Test (++); Baljet Test (++); Legal's Test (+) indicated the presence of anthroquinone glycosides⁴²; Test for Cardiac Glycosides in the leaf extracts of CI using Keller-Killani Test (++) indicated the presence of cardiac glycosides; Test for Carbohydrates in the leaf extracts of CI using Molish's Test (++) Fehling's Solution Test (+); Benedict's Reagent Test (++) presence of carbohydrates in the CIMLE⁴¹; Test for Phytosterol in the leaf extracts of CI using Libermann Burchard's (+) and Salkowski's Test (+) depicted the presence of phytosterols in the leaf samples of CI⁴³; Test for Flavonoids in the leaf extracts of CI using Ferric Chloride Test (++) and Shinod's Test (++) showed the presence of flavonoids as indicated by Reddy et al.⁴⁴; Test for Fixed Oils and Fats in the leaf extracts of CI Spot Test (+); Saponification (+); Test for Free Amino Acids in the leaf extracts of CI Million's Reagent (++); Ninhydrin Reagent (++) Test for Tannins in the leaf extracts of CI using 5% Ferric Chloride (-) and 10% Lead Acetate (-) showed no results indicating the absence of tannins in the sample; Test for Saponins in the leaf extracts of CI Foam Test (+) indicated the presence of saponins; however, gums & mucilage were absent in CIMLE samples (Table 1). In the present study, phytochemical screening of methanolic leaf extracts of *C. igneus* showed the presence of alkaloids, glycosides, saponins, phytosterols, phenolics, and carbohydrates as indicated earlier by Khanday et al⁴⁵.

GCMS profile of *Costus igneus* indicated the presence of 21 compounds in the sample. At RTI of 16.303 Bicyclo [3.1.1]heptane, 2,6,6- trimethyl- (C₁₀H₁₈); at RTI of 16.361 - 2-Pentadecanone, 6,10,14-trimethyl- (C₁₈H₃₆O); at RTI of 16.564 - Bicyclo [3.1.1]heptane, 2,6,6- trimethyl- (C₁₀H₁₈); at RTI of 16.637 phthalic acid (C₈H₆O₄); at RTI of 16.739 - 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (C₂₀H₄₀O); at RTI of 17.145 - 5,9,13-Pentadecatrien-2-one, 6,10,14- trimethyl (C₁₈H₃₀O); at RTI of 17.595 - 2(3H)-Furanone,dihydro-5-methyl-5- pentyl (C₁₀H₁₈O₂); at RTI of 17.828 - Hexamethyl-2,6,10,14,18,22-tetracosahexaen-1-yl]-6-methox (C₃₀H₅₀O); at RTI of 18.525 - Oxalic acid, cyclohexyl isohexyl ester (C₁₄H₂₄O₄); at RTI of 18.844 - 6-Octadecenoic acid (C₁₈H₃₄O₂); at RTI of 19.077 - Phytol (C₂₀H₄₀O); at RTI of 21.066 - 4,8,12,16-Tetramethylheptadecan-4- olide (C₂₁H₄₀O₂); at RTI of 21.313 - 5-methyl-2,3-dihydro-1H-indole (C₉H₇BrN₂O); at RTI of 22.591 - Bis(2-ethylhexyl) phthalate (C₁₂H₁₄O₄); at RTI of 24.232 - Adamantane-1-carboxylic acid methyl ester (C₁₂H₁₈O₂); at RTI of 25.815 - Benzopyran-6-ol,3,4-dihydro- (C₂₂H₃₆O₂); at RTI of 25.815 - Benzoquinoline (C₁₃H₉N); at RTI of 26.193 - gamma-Tocopherol (C₂₈H₄₈O₂); at RTI of 26.411 - dimethyl-5,5'-diphenyl-1H, (C₁₄H₇NO₃); at RTI of 28.56 - 1H-benzo[b]furo[2,3-f]indole (C₁₈H₁₆N₆S₂) were detected in the samples^{46,47} (Table 2; Fig. 1a-l). IUPAC name and 2D structure of bioactive compounds in *Costus igneus* have been listed in Table 3. Further, FTIR analysis confirmed the presence of the reported compounds in the samples (Table 4; Fig.2). Recently, Adetayo and Anyasor⁴⁸ through *in-silico* approach demonstrated gastroprotective activity of bioactive compounds from n-butanol fraction of *Costus igneus* on antiulcer druggable targets and showed that NQP exhibited substantial inhibitory effects on proton pump, urease activity, muscarinic and histamine receptors.

CONCLUSION

In the present study, both phytochemical screening and GCMS/ FTIR analysis of CIMLE showed the presence of as much as 20 bioactive compounds in the methanolic leaf extracts. If further explored for potential source of drug leads under deeper dimensions of ADMETox, this traditional medicinal plant could provide novel leads for the management of diabetics and its associated complications.

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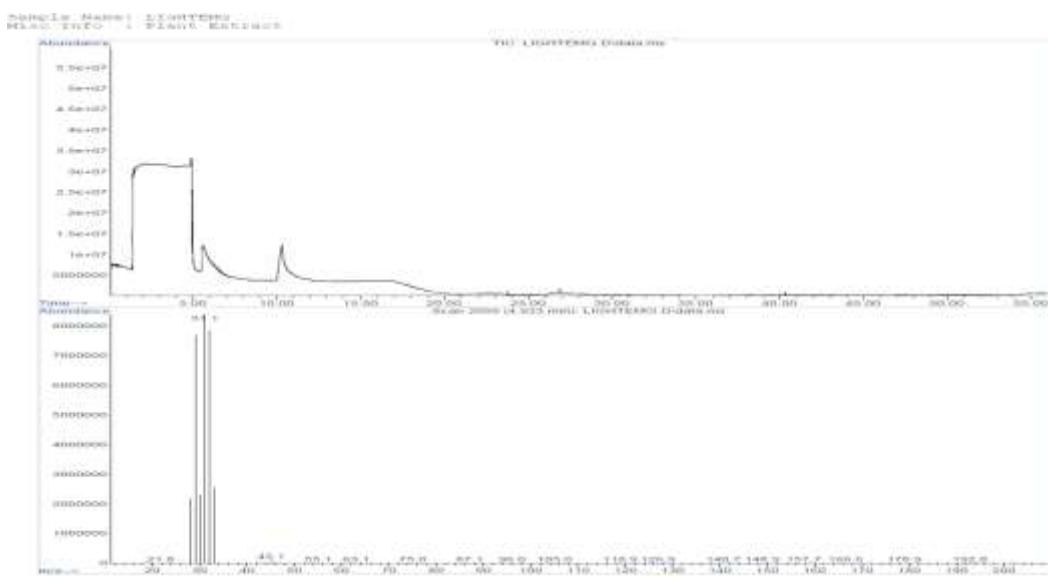
Table 1 Phytochemical Examination with solvent plant extracts

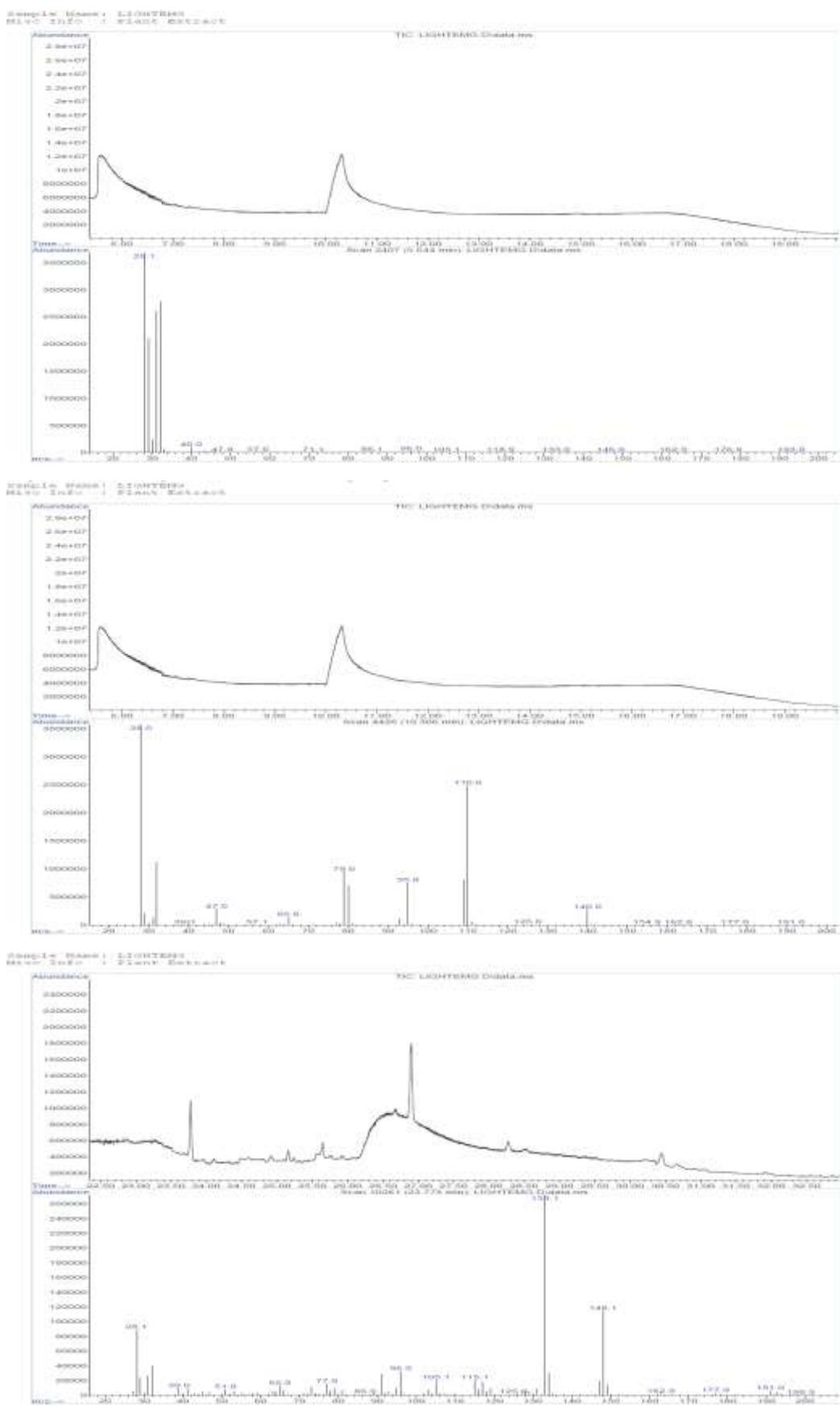
S. No	Plant constituents tested & Reagent used	Methanol Extract (CIMLE)
1	TEST FOR ALKALOIDS	
1.1	<i>Mayer's test</i>	+++
1.2	<i>Dragendorff's test</i>	+++
1.3	<i>Hager's test</i>	+++
1.4	<i>Wagner's test</i>	+++
2.1	TEST FOR GLYCOSIDES - Anthroquinone	
2.1.1	<i>Borntrager's test</i>	++
2.1.2	<i>Baljet test</i>	++
2.1.3	<i>Legal's test</i>	+
2.2	TEST FOR GLYCOSIDES - Cardiac	
2.2.1	<i>Keller-Killani test</i>	++
3	TEST FOR CARBOHYDRATES	
3.1	<i>Molish's test</i>	++
3.2	<i>Fehling's solution test</i>	+
3.3	<i>Benedict's reagent test</i>	++
4	TEST FOR PHYTOSTEROLS	
4.1	<i>Liebermann Burchard's</i>	++
4.2	<i>Salkowski's test</i>	+
5	TEST FOR FLAVONOIDS	
5.1	<i>Ferric chloride test</i>	+
5.2	<i>Shinod's test</i>	++
6	TEST FOR FIXED OILS AND FATS	
6.1	<i>Spot test</i>	+
6.2	<i>Saponification</i>	+
7	TEST FOR FREE AMINO ACIDS	
7.1	<i>Million's reagent</i>	++
7.2	<i>Ninhydrin reagent</i>	++
8	TEST FOR TANNINS	
8.1	<i>5% Ferric chloride</i>	-
8.2	<i>10% Lead acetate</i>	-
9	TEST FOR SAPONINS	
9.1	<i>Foam test</i>	+
10	GUMS & MUCILAGE	-

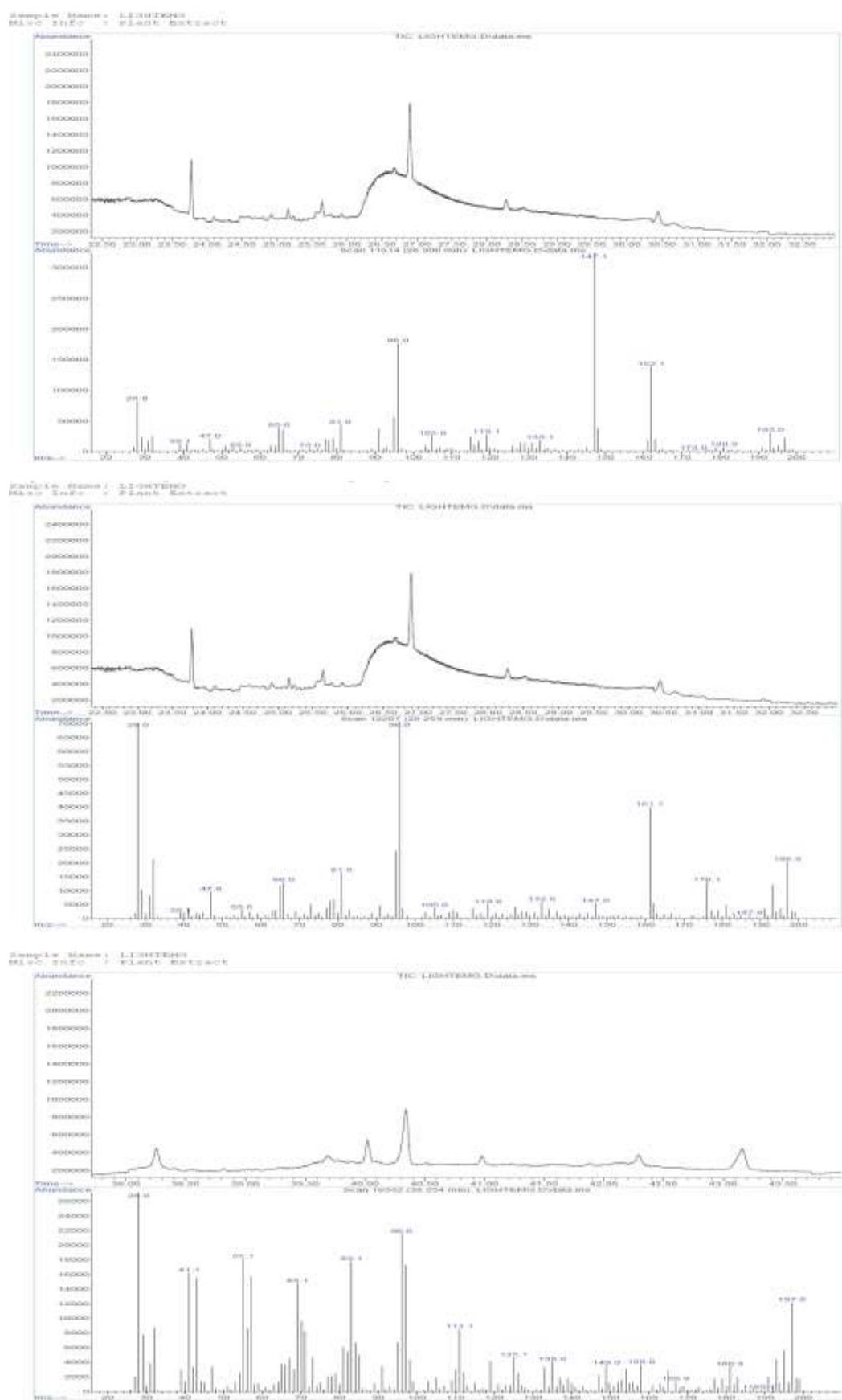
Note: + indicates positive result; - indicates negative result

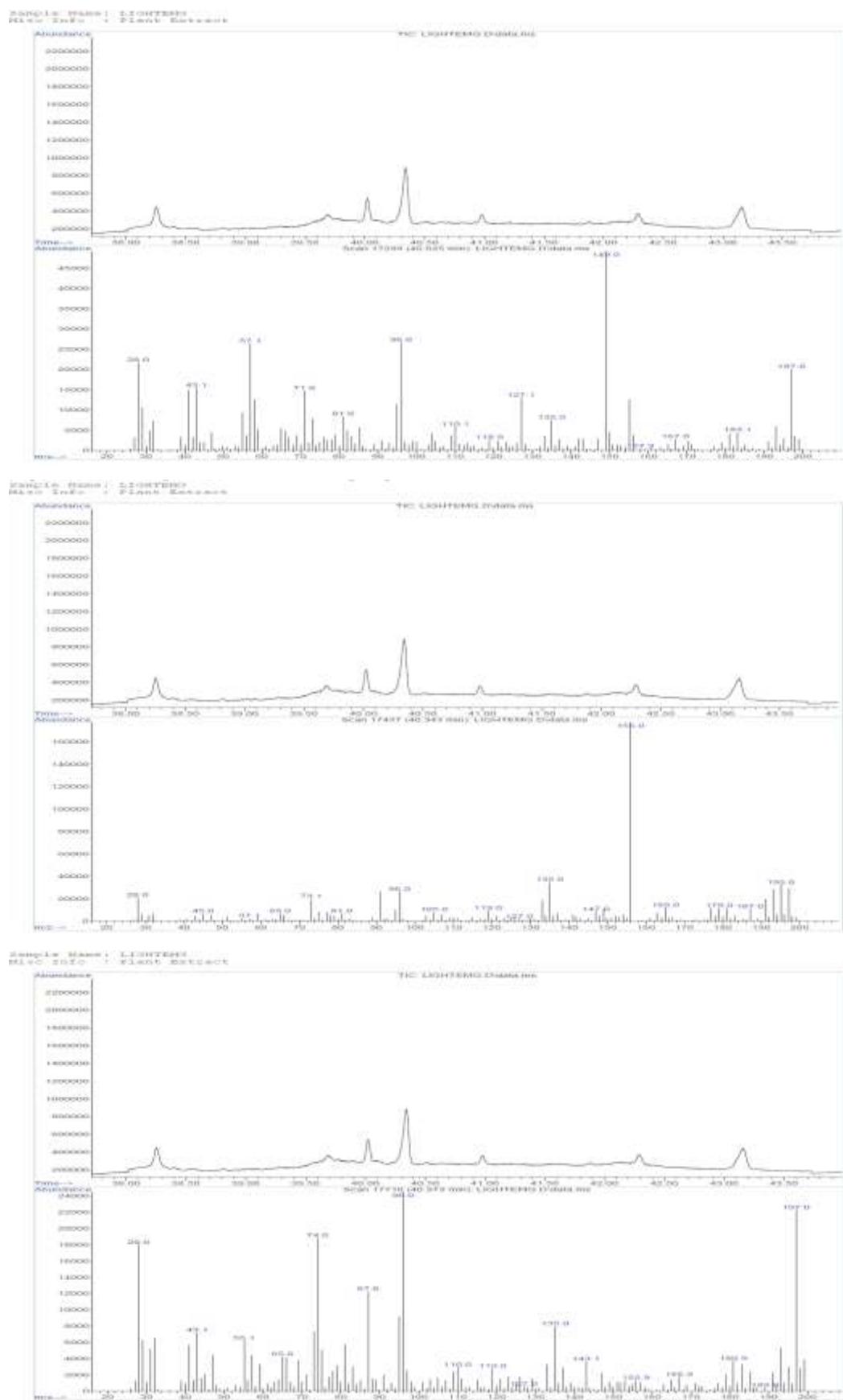
Table 2 GCMS Profile of Bioactive Compounds in *Costus igneus*

RT	COMPOUND	MW	MF	AREA%
16.303 ^a	Bicyclo [3.1.1]heptane, 2,6,6- trimethyl-	138.24	C ₁₀ H ₁₈	4.27
16.361 ^b	2-Pentadecanone, 6,10,14-trimethyl-	268.47	C ₁₈ H ₃₆ O	6.68
16.564	Bicyclo [3.1.1]heptane, 2,6,6- trimethyl-	138.24	C ₁₀ H ₁₈	0.79
16.637	phthalic acid	166.13	C ₈ H ₆ O ₄	0.34
16.739 ^c	3,7,11,15-Tetramethyl-2-hexadecen- 1-ol	296.54	C ₂₀ H ₄₀ O	2.43
17.145 ^d	5,9,13-Pentadecatrien-2-one, 6,10,14- trimethyl	262.41	C ₁₈ H ₃₀ O	14.33
17.595 ^e	2(3H)-Furanone,dihydro-5-methyl-5- pentyl	170.24	C ₁₀ H ₁₈ O ₂	3.92
17.828	Hexamethyl-2,6,10,14,18,22- tetracosahexaen-1-yl]-6-methox.	426.71	C ₃₀ H ₅₀ O	0.82
18.525	Oxalic acid, cyclohexyl isohexyl ester	256.33	C ₁₄ H ₂₄ O ₄	0.83
18.844	6-Octadecenoic acid	282.46	C ₁₈ H ₃₄ O ₂	0.77
19.077 ^f	Phytol	296.53	C ₂₀ H ₄₀ O	9.52
21.066 ^g	4,8,12,16-Tetramethylheptadecan-4- olide	324.54	C ₂₁ H ₄₀ O ₂	5.37
21.313	5-methyl-2,3-dihydro-1H-indole	239.08	C ₉ H ₇ BrN ₂ O	0.64
22.591	Bis(2-ethylhexyl) phthalate	222.24	C ₁₂ H ₁₄ O ₄	1.05
24.232	Adamantane-1-carboxylic acid methyl ester	194.27	C ₁₂ H ₁₈ O ₂	1.04
25.815 ^h	Benzopyran-6-ol,3,4-dihydro-	332.51	C ₂₂ H ₃₆ O ₂	3.37
25.815 ⁱ	Benzoquinoline	179.23	C ₁₃ H ₉ N	1.33
26.193 ^j	gamma-Tocopherol	416.68	C ₂₈ H ₄₈ O ₂	4.96
26.411 ^k	dimethyl-5,5'-diphenyl-1H,	237.21	C ₁₄ H ₇ NO ₃	15.05
28.56 ^h	1H-benzo[b]furo[2,3-f]indole	380.5	C ₁₈ H ₁₆ N ₆ S ₂	20.57









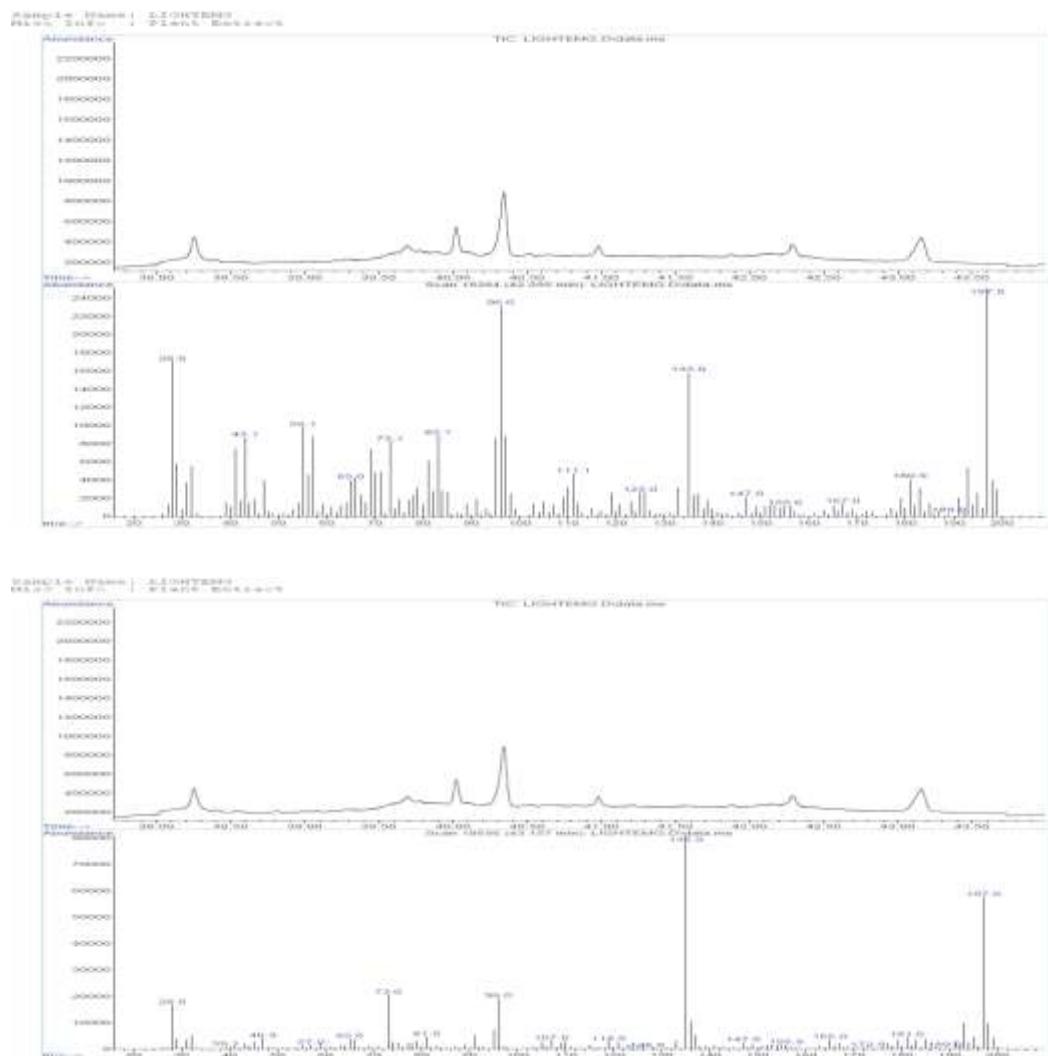
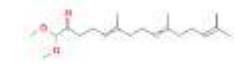
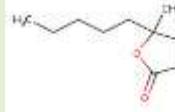
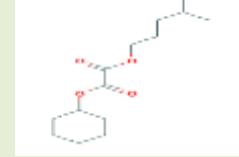
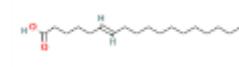
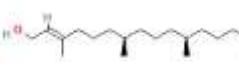
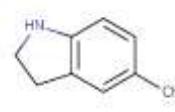
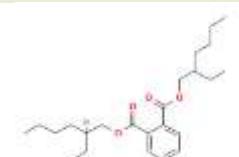
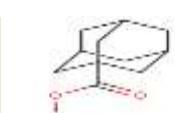
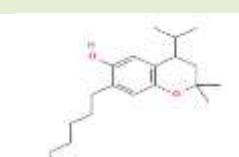
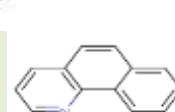
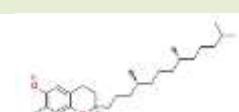
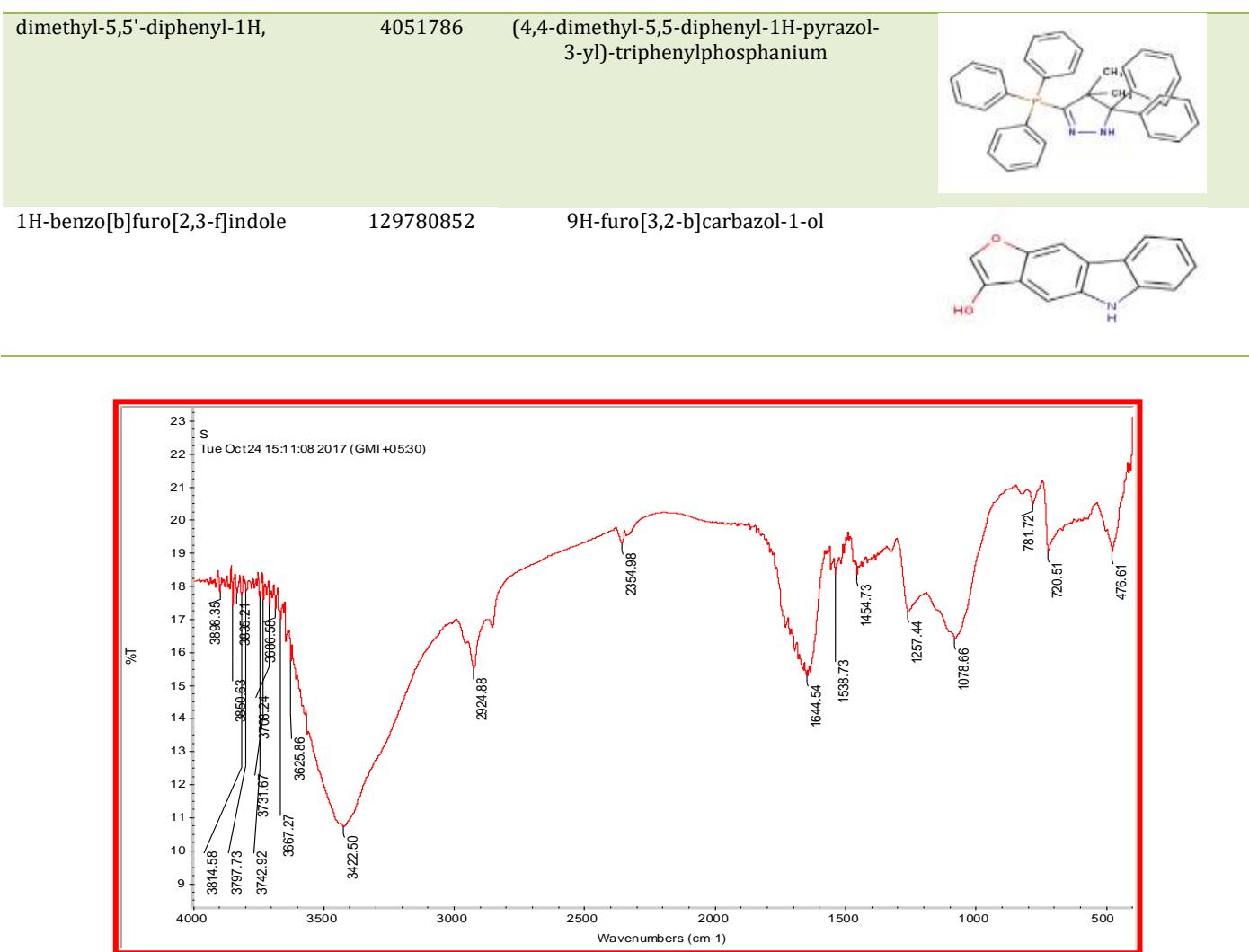


Fig. 1(a-l) GC-MS profile of bioactive compounds in *Costus igneus*

Table 3 IUPAC name and 2D structure of bioactive compounds in *Costus igneus*

COMPOUND	CID	IUPAC NAME	2D STRUCTURE
Bicyclo [3.1.1]heptane, 2,6,6-trimethyl-	12314300	(1S,2R,5R)-2,6,6-trimethylbicyclo[3.1.1]heptane	
2-Pentadecanone, 6,10,14-trimethyl-	10408	6,10,14-trimethylpentadecan-2-one	
Bicyclo [3.1.1]heptane, 2,6,6-trimethyl-	12314300	(1S,2R,5R)-2,6,6-trimethylbicyclo[3.1.1]heptane	
Phthalic Acid	1017	Phthalic Acid	

3,7,11,15-Tetramethyl-2-hexadecen- 1-ol	5366244	(E)-3,7,11,15-tetramethylhexadec-2-en-1-ol	
5,9,13-Pentadecatrien-2-one, 6,10,14- trimethyl	53946054	1,1-dimethoxy-6,10,14-trimethylpentadeca-5,9,13-trien-2-one	
2(3H)-Furanone,dihydro-5-methyl-5- pentyl	103702	Dihydro-5-methyl-5-pentylfuran-2(3H)-one	
Hexamethyl-2,6,10,14,18,22-tetracosahexaen-1-yl]-6-methox.	57417215	2,6,10,14,18,22-Tetracosahexaene	
Oxalic acid, cyclohexyl isohexyl ester	6421306	2-O-cyclohexyl 1-O-(4-methylpentyl) oxalate	
6-Octadecenoic acid	5282754	(E)-octadec-6-enoic acid	
Phytol	5280435	(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	
4,8,12,16-Tetramethylheptadecan-4- olide	567149	5-methyl-5-(4,8,12-trimethyltridecyl)oxolan-2-one	
5-methyl-2,3-dihydro-1H-indole	14023679	5-methyl-2,3-dihydro-1H-indole	
Bis(2-ethylhexyl) phthalate	8343	bis(2-ethylhexyl) benzene-1,2-dicarboxylate	
Adamantane-1-carboxylic acid methyl ester	136553	methyl adamantane-1-carboxylate	
Benzopyran-6-ol,3,4-dihydro-	91221	2,2-dimethyl-7-octyl-4-propan-2-yl-3,4-dihydrochromen-6-ol	
Benzoquinoline	9191	benzo[h]quinoline	
gamma-Tocopherol	92729	(2R)-2,7,8-trimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-3,4-dihydrochromen-6-ol	

Fig. 2 FTIR spectra of bioactive compounds in *Costus igneus*Table 4 FTIR profile of bioactive compounds in *Costus igneus*

Absorption Range (cm ⁻¹)	Frequency Range (cm ⁻¹)	Appearance of the Peak	Participatory Function Group	Compound Class
3898.26	3900-3584	Medium, Sharp	O-H Stretching	Alcohol
3850.62	3900-3584	Medium, Sharp	O-H Stretching	Alcohol
3835.10	3900-3584	Medium, Sharp	O-H Stretching	Alcohol
3742.73	3900-3584	Medium, Sharp	O-H Stretching	Alcohol
3731.73	3900-3584	Medium, Sharp	O-H Stretching	Alcohol
3708.45	3900-3584	Medium, Sharp	O-H Stretching	Alcohol
3686.55	3900-3584	Medium, Sharp	O-H Stretching	Alcohol
3667.59	3900-3584	Medium, Sharp	O-H Stretching	Alcohol
3625.96	3900-3584	Medium, Sharp	O-H Stretching	Alcohol
3419.82	3400-3300	Medium	N-H Stretching	Aliphatic Primary Amine
2924.35	3000-2840	Medium	C-H Stretching	Alkane
2357.90	2400-2000	Strong	O=C=O Stretching	Carbon Dioxide
1644.76	1648-1638	Strong	C=C Stretching	Alkene
1556.02*	1550-1500	Strong	N-O Stretching	Nitro Compound
1538.93	1550-1500	Strong	N-O Stretching	Nitro Compound
1454.87	1465-1450	Medium	C-H Bending	Alkane
1077.64	1250-1020	Medium	C-N Stretching	Amine
720.50	840-790	Medium	C=C Bending	Alkene
478.53*	750 ± 20	--	--	Benzene Derivative