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Comparative Study on the Phytochemical Analysis of Costus spicatus (Jacq.) Leaf and Rhizome

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Abstract

Costus spicatus, an herbaceous plant, parts- leaves, rhizome, stem and root are used locally because of its medicinal properties. This study was carried out to compare the phyto-constituents of the Costus spicatus leaf and rhizome. The secondary metabolites of Costus spicatus leaf and rhizome were determined qualitatively via standard phytochemical screening methods. The chemical compositions of the aqueous extract of Costus spicatus leaf and rhizome were analyzed using Gas Chromatography-Mass Spectrometry and UV-Visible Spectrophotometer. The qualitative phytochemical analysis revealed the presence of Carbohydrate, Alkaloids, Saponins, Terpenoids, Phenols and Protein were detected in the aqueous rhizome extract while Carbohydrate, Alkaloids, Flavonoids and Proteins were detected in the leaf extract. The UV-yis scan of the aqueous rhizome and leaf extracts revealed the varying absorbance of the leaf and rhizome extract at different wavelengths and the spectrum shows the highest peaks at 200-214 nm and 220-227 nm respectively indicating the presence of organic chromophores. The GC-MS analysis of aqueous extract of Costus spicatus rhizome revealed the presence of Hexadecanoic acid, methyl ester (11.34%) and 10,13-Octadecadienoic acid, methylester (10.22%) for the leaf extract as the compound with the highest peaks. These results reveal that the rhizome extract contains more active organic compounds and therefore can be further explored in phytomedicinal researches.

Keywords: Costus spicatus, phytochemical analysis, UV-spectroscopy, GC-MS analysis.

Introduction

Plant chemistry has advanced in recent years as a diverse discipline between natural product, organic chemistry and plant biochemistry. Plants with an ethnobotanical history are known to harbor diverse group of chemicals, which constitute major natural sources of bioactive compounds with the chemical structures, biosynthesis and metabolism of these substances as well as their natural distribution and their biological function. (Ajayi, 2019). Costus spicatus is a perennial herb known commonly as spiked Spiralhead ginger or Insulin plant in English, Tete-egun in yoruba, Okpete in igbo and Kakizuwa in hausa There are more than 100 species of the Costus. A few assortments with flowers and bracts look like conservative cones, while others are molded like pineapple or delicate crepe emerging from green cones. A few leaves on the abaxial surface are pubescent, although most are glossy and purplish. There are about seven members in the Costus Linn family established from India. Other developed species of this genus are C.barbatus, C.chartaceus, C. cuspidatus, C. giganteus, C. igneus, C. spectabilis and C.Pictus (Jena et al.,

2016). The genus Costus spicatus belongs to the family Costaceae. They are perennial tropical plants. They are often distinguished from plants of the genus Zingiber the spiraling growth of their stems. Costus spicatus presents substituting leaves, membranous, Papyraceous provided with sheaths, smooth on the two sides, 25-40 cm long and 6-10 cm wide. It has strobili-shaped inflorescences in terminal spikes, with large showy red bracts, which shield the yellowish flowers. Multiply both by seeds and by rhizomes. Costus spicatus Sw. (Costaceae) is a conspicuous herb utilized by Dominicans in the Dominican Republic and the US for the treatment of diabetes, a developing scourge in the Hispanic people group (David et al., 2021).

Although the utilization of herbs for medicinal purposes have diminished recently as a result of growing knowledge and civilization, the fact still remains that they are still used in other parts of the world. In Nigeria and other third world countries, a number of people still practice traditional medicine as a result of their perception, standard of living, and cultural background amongst other factors (Olutayo et al., 2021). It has been affirmed scientifically that different plant parts such as roots, stems, rhizome and leaves contain phytochemicals which are the bioactive non-nutritive chemical components with a potential to reduce the risk of chronic diseases, and serve certain nutritional purposes (Sandra et al., 2018; Elke and Emanuele, 2013). Richard et al. (2021) reported that plant's mechanism of defence against micro and macro predation results from the plant's ability to synthesize to a limitless ability, aromatic compounds which are mostly secondary metabolites. Over the years in Nigeria, there has been a debate as to the activity and effectiveness in treatment of illnesses and pathogens between the leaves and rhizomes of the medicinal plants - Costus spicatus- amongst various practitioners and patronizers of traditional medicine. It is therefore pertinent to conduct a comparative analysis on the phytochemical components of the Costus spicatus leaves and rhizome to infer scientifically which of them will be more active and effective in traditional medical purposes.

With increasing advance in research, the use of analytical techniques has unleashed the use of TLC, UV, NMR and GC-MS in separation, identification and structural determination of phytochemicals in plant (Altemimi et al., 2017). The aim of this study is to compare the phytocontituents present in the Costus spicatus leaf and rhizome extracts using phytochemical screening, UV-spectrophotometry and GC-MS Techniques to provide further insight of the use of C. spicatus

leaf and rhizome in tradition medicine and drug discovery.

Material and Methods

Fresh leaves and rhizomes of the Costus Spicatus plant were collected at the Mountain Top University Campus premises, Prayercity, Ibafo, Ogun State, Nigeria. The plant was identified at the University of Lagos Botany Department, where a voucher specimen (Number 8571) was prepared and deposited.

Preparation of Aqueous Extract

The identified samples were thoroughly rinsed under running water to remove contaminants, oven dried at 50 degree Celsius to a constant weight and pulverized to powder using an electric blender. The rhizome and leaf (350 g) powder were weighed and soaked separately in 2600 ml of distilled water for 48 hours and later sieved and filtered using a Watman's filter paper no 1. The rhizome and leaf filtrate were collected in beakers and lyophilized to yields of 47.57 g and 41.98 g (15.85 and 13.22 % yield) respectively. The concentrates were then stored in a refrigerator at ?4°C for further analysis.

Qualitative Phytochemical analysis

The aqueous flower and leaf extract were screened for the presence of secondary metabolites using standard methods as described by Trease and Evans (1989).

GC-MS Analysis

The GC-MS research was carried out using a Hewlett Packard Gas Chromatograph (Model 6890 series) fitted with a Hewlett Packard 7683 series flame ionization detector and a 250 °C MS transfer line temperature injector. A capillary fused silica column- HP-5MS (30 x 0.25 mm), 1.0 ?m film thickness, was mounted to the GC. The oven temperature was held for 5 min of holding time at 50 ° C and raised from 50 to 250 °C at a rate of 2 °C / min, using helium gas (99.999 percent) At a steady flow rate of 22 cm / s as a carrier gas. At a 1:30 split ratio, 1.0 microns of extract was injected (1 mg dissolved in 1 ml of absolute alcohol). Mass Spectrometer Agilent Technology Network (Model 5973 series) coupled with Hewlett Packard Gas Chromatograph (Model 6890 series) with NIST08 Library software database was analyzed by MS At 70 eV/200 °C, 1 scan/s scanning rate, mass spectra were taken. Using the NIST08 Library database, compound recognition was performed. The mass spectrum of unknown individual compounds was compared with the compounds found in the database of the Library software (Corley et al., 2005).

UV-spectrophotometry

The absorbance and wavelength of the peaks were determined for the aqueous extract of the leaf and rhizome extract by a wavelength scan

between 200 and 227 nm (Rice-Evans and Miller, 1996). UV-spectra were reported on a UV- spectrophotometer (Shimadzu UVd-1800 PC, Japan).

Results and Discussion

Yeum and Russell (2014) reported that the qualitative analysis of the phytochemical component of aqueous plant extracts is employed generally to determine, majorly, the antioxidant function as well as the potential medicinal benefit of the plant. Although the human system performs anti-oxidant activities of mopping up free-radicals naturally, these reactive species can also be mopped by exposure of the cell to chemical agents. Due to the presence of unpaired valent electrons, generated free radicals are very reactive and unstable enabling them to destroy the membrane structure of cell and its constituents, thus causing diseases (Somade et al., 2020). The phytochemical constituent of the different plant parts in this study indicates the overall antioxidant potentials of the parts. From our findings (Table 1), Costus spicatus leaves extract has a similar phytochemical constituent to its rhizome except for the absence of phenols, terpenoid and saponin. These phenolic compounds, terpenoids and saponins possess antioxidant and antimicrobial properties (El-Aziz et al., 2019; Brahmkshatriya and Brahmkshatriya, 2013; Jin and Russell, 2010). Similarly, Himaja et al., 2010 reported that the rhizome of Curcuma zedoaria showed potential anti-oxidant property compared to other region of the plant as it contained terpenoids, saponins and flavonoids. Both plant parts indicates the absence of anthraquinine which also is an antioxidant and antimicrobial agent. Results from this study infers that the rhizome of Costus spicatus may be more effective in counteracting free-radical and as it shows a more potential anti-oxidant activity.

The absorbance of a solution is a measure of the amount of light that a solution absorbs at a particular wavelength. The absorption maximum wavelength may be used to indicate the prescence of certain compounds and biomolecules in a solution. In this study, the absorbance value of the extract solution was observed in the wavelengths range, 200-230 nm; the maximum absorbance of the leaves and rhizomes extracts were noted at ranges 200-214 nm and 220-227 nm respectively (Figures 1 and 2). As a confirmatory to the previous phytochemical assessment presented (Table 1), the absorbance maximum wavelength identifies the secondary metabolites present within the wavelength range observed aforementioned (Table 2). The value of the wavelength range of the rhizome extract of Costus spicatus correlates with the absorption maximum wavelength

values of the Saponins, Terpenoids and Proteins as reported by El-Barky et al. (2016), Baas and Neimann (1978), and Layne (1957), but was not represented in the leave's. Since sarponin and terpenoid possess antioxidant and antimicrobial activity (Brahmkshatriya and Brahmkshatriya, 2013; Jin and Russell, 2010), then the result indicates that the rhizome of Costus spicatus may be more useful in herbal medicine administration for illness treatment.

In identifying the bioactive compounds in plants, the GC-MS is considered a proven technique as it suggests a plant's possible curative properties (Uraku, 2015). In compound detection, the GC-MS is used to separate, quantify, and identify the analytes present in plant samples (Maurer, 1995). In this study, the GC-MS analysis of the aqueous extract of Costus spicatus leaves and rhizomes identify the presence of a number of organic compounds. Eight constituents were predominantly found in the aqueous rhizome extract of the plant (Figure 3). These constituents and their calculated peak area compositions included hexadecanoic acid, methyl ester (11.34%), 9, 12-octadecadienoic acid, methyl ester (11.21%) cyclononasiloxane, octadecamethyl (1.49%), carbonic acid, but-3-en-1-yl penta decyl ester (1.62%) cyclononasiloxane, octadecamethyl (2.11%), 1,2,4, triazolo1,5-apyrimidine-6 -carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester (2.47%) cyclononasiloxane, octadecamethyl (3.28%) and cyclohexane, 1,1'-(2-methyl-1,3 propanediyl bis (18.66%). Most of these constituents have been found to show interesting biological activity against certain illnesses and pathogens. For instance, the anti-oxidant (Reuben et al., 2021), antifungal (Chandrasekaran et al., 2011), antiinflammatory (Aparna et al., 2012), activities reported for hexadecanoic acid, methyl ester and the antimicrobial, antioxidant and antitumor (Alkhamis et al., 2021) reported for cyclohexane, 1,1'-(2-methyl-1,3 propanediyl)bis, may suggest the rationale for the traditional use of the species.

Ten volatile phytochemical constituents were found to be abundant in the aqueous leaves extract of Costus spicatus (Figure 4). These constituents and their calculated percentage peak area compositions included 1-decyne (1.73%), heptanoic acid, ethyl ester (1.51%), hepta-4,6-dienoic acid`, ethyl este-1-Octyne (3.53%), diethyl azelate (2.26%), hexadecanoic acid, methyl ester (4.95%), hexadecanoic acid, methyl ester (1.37%), 10,13-octadecadienoic acid, methylester (10.22%), bicyclo[4.1.0]heptane, 3-methyl (2.75%), (E,E,Z)-1,3,12-nonadecatriene-5,14-diol (5.55%) and 1-methyl-2-methylenecyclohexane (4.46%). Most of these constituents have been found to show interesting biological activity

against certain microbes. The anti-microbial activity reported for the abundant constituents present in the leave extract from the GC-MS analysis- E,E,Z-1,3,12-Nonadecatriene-5,14-diol (Tian, 2019; Zhang et al., 2016) and 10,13-Octadecadienoic acid, methylester (Abubakar and Majinda, 2016) suggests its potential suitability for pathogenic-purposes.

The compound hexadecanoic acid, methyl ether was found in both extracts. It may be inferred that both parts of the plant may have potential effectiveness against illnesses such as skin diseases, hair infections and respiratory conditions and as anti-inflammatories and analgesics due to the presence of hexadecanoic acid, methyl ether. However, this result (Table 3) infers that the Costus spicatus rhizome extract may have a more pronounced antioxidant, anti-inflammatory, anti-cancer, and anti-fungal potentials compared to the leave extract. Also, considering the abundance of the compounds presently, we infer that the leave extract of Costus spicatus may have potential anti-microbial (such as anti-fungal and anti-bacterial) activities.

The GC-MS analysis showed the presence of eighteen phytochemical constituents from the aqueous polar extracts of the rhizome and leaves of Costus spicatus. From the GC-MS and other analysis conducted, we therefore can conclude that both plant parts possess an anti-microbial activity but the rhizome of the Costus spicatus may be more effective in traditional treatment of illnesses and pathogenic infections as it has a great antioxidant and antimicrobial potential; it is also associated with the presence of the functional phytochemicals (hexadecanoic acid, methyl ether; cyclohexane, 1,1'-(2-methyl-1,3 propanediyl)bis; terpenoids, saponins, flavonoids, and phenols). Each compound identified has its own biological importance and further study of the plant phytochemicals by in silico and in vitro methods may further prove its medicinal importance.

Table 1: Qualitative analysis of aqueous extract of *Costus spicatus* leaves and rhizome

Secondary Metabolites	Leaves	Rhizome
Carbohydrate	++	++
Phenols	- -	++
Tannin	- -	- -
Alkaloids	++	++
Flavonoids	++	++
Proteins	++	++
Terpenoids	- -	++
Anthraquinones	- -	- -
Saponin	- -	++

Present ++; Absent - -

Table 2: The wavelength of maximum absorbance of the various screened phytochemicals

Secondary Metabolites	Absorption Maximum Wavelength	References
Carbohydrate	384 nm	Jayanta *et al.* (2021)
Phenols	280 nm	Maya *et al.* (2021)
Tannin	250-450 nm	Leanna and Jason (2007)
Alkaloids	418 nm	Luyang *et al.* (2014)
Flavonoids	408 nm	Ramos *et al.* (2017)
Protein	200 nm for peptide bonds and 280 nm for aromatic amino acids	Layne (1957), and Stoscheck (1990)
Terpenoids	220 nm	Baas and Neimann (1978)
Anthraquinones	328 -505 nm	Anouar *et al.* (2014)
Saponin	218-282 nm	El-Barky *et al.* (2016), and Maria *et al.* (2011)

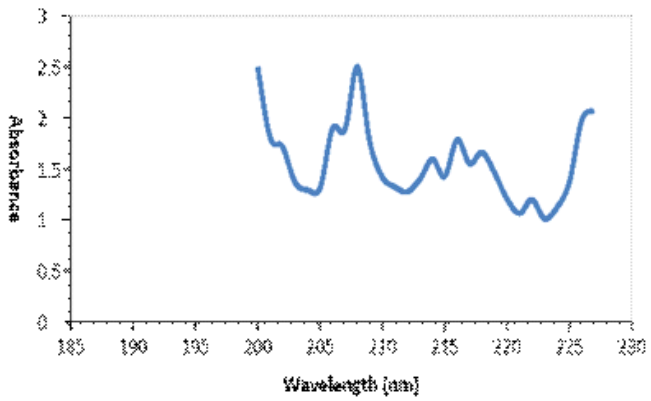

Fig. 1: Ultra violet -Spectroscopy of *Costus spicatus* rhizome

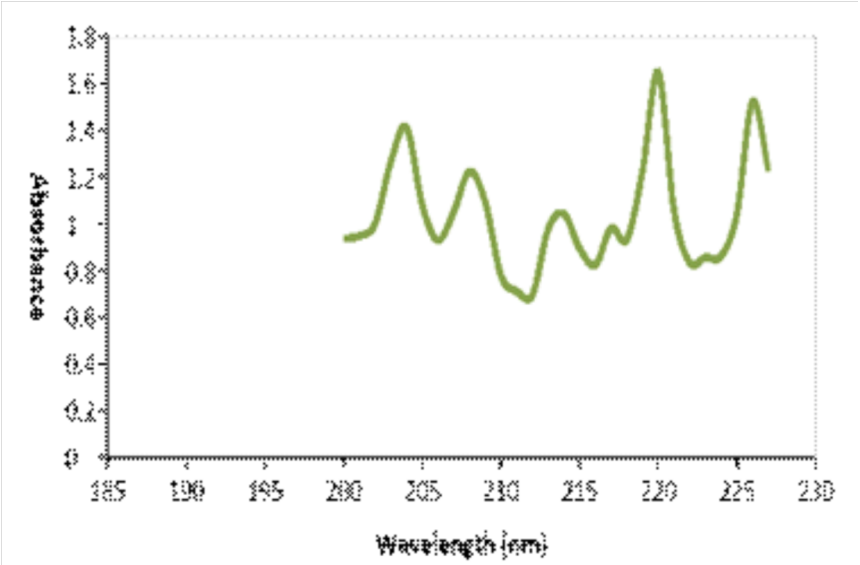

Fig. 2: Ultra violet -spectroscopy of *Costus spicatus* leaves

Table 3: Phytochemical components identified by GC-MS in aqueous extract of *C. spicatus* rhizome and leave extract

Plant part	Compound Name	Mol. Formular	Mol. Wt. (g/mol)	RT (min)	Peak Area (%)	Reported Bioactivity
Costus spicatus rhizome extract	Hexadecanoic acid, methyl Ester	$C_{17}H_{34}O_2$	270.5	15.279	11.34	Antioxidant (Reuben *et al.,* 2021), Anti - bacteria and Anti-fungal (Chandrasekaran *et al.,* 2011), Anti-inflammatory (Aparna *et al.,* 2012)
	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294.5	16.624	13.21	Anti-microbial and anti -oxidant activity (Nuerxiati *et al.,* 2021), and Anti-cancer (Yu *et al.,* 2005)

Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	667.4	21.510	1.49	Anticancer (Lutfia *et al.* , 2021; Babalola, 2020), Anti -fungal (Lotfi *et al.,* 2021), and Anti-bacteria (Hussin *et al.,* 2021)
Carbonic acid, but -3-en-1-yl penta decyl ester	$C_{15}H_{28}O_3$	326.5	21.868	1.62	Antioxidant, analgesic, and anti-inflammatory effects (Núñez *et al.,* 2021)
Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	667.4	22.278	2.11	Anti-fungal (Lotfi *et al.,* 2021), Anticancer (Lutfia *et al*., 2021), and Antibacterial and anti -fungal (Hussin *et al.,* 2021)
1,2,4-triazolo (1,5-a) pyrimidine-6-carboxylic acid, 4,7 -dihydro-7-imino, ethyl ester	$C_{10}H_{12}N_4O_3S$	268.3	22.758	2.47	Anticancer (Dockerill *et al.,* 2021) and antimicrobial *(Nasri et al., 2021)*
Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	667.4	23.017	3.28	See above
Cyclohexane, 1,1' -(2-methyl-1,3 propanediyl)bis-	$C_{16}H_{30}$	222.4	28.643	18.66	Antimicrobial, antioxidant and antitumor (Alkhamis *et al.*, 2021)
1-Decyne	$C_{10}H_{18}$	138.3	5.790	1.73	Metabolite (NCBI, 2021a)
Heptanoic acid, ethyl ester	$C_9H_{18}O_2$	158.2	9.134	1.51	Hemolytic, Pesticidal, and anti-oxidant (Femi-Adepoju *et al.*, 2021)
Hepta-4,6-dienoic acid, ethyl este-1-Octyne	$C_9H_{14}O_2$	154.2	10.554	3.53	No information
Diethyl azelate	$C_{11}H_{20}O_4$	244.3	13.217	2.26	Food Additive (NCBI, 2021b)
Hexadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	270.5	15.267	4.95	See above

Hexadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	270.5	15.400	1.37	See above
10,13-Octadecadienoic acid, methylester	$C_{19}H_{34}O_2$	294.5	16.676	10.22	Anti-microbial (Abubakar and Majinda, 2016)
Bicyclo[4.1.0]heptane, 3-methyl-	C_8H_{14}	110.2	17.046	2.75	Antibacterial (Anand *et al.,* 2021)
E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	294.5	17.219	5.55	Antimicrobial (Tian, 2019; Zhang *et al.,* 2016)
1-Methyl-2-methylenecyclohexane	C_8H_{14}	110.2	17.727	4.46	No information
1-Decyne	$C_{10}H_{18}$	138.3	5.790	1.73	See above

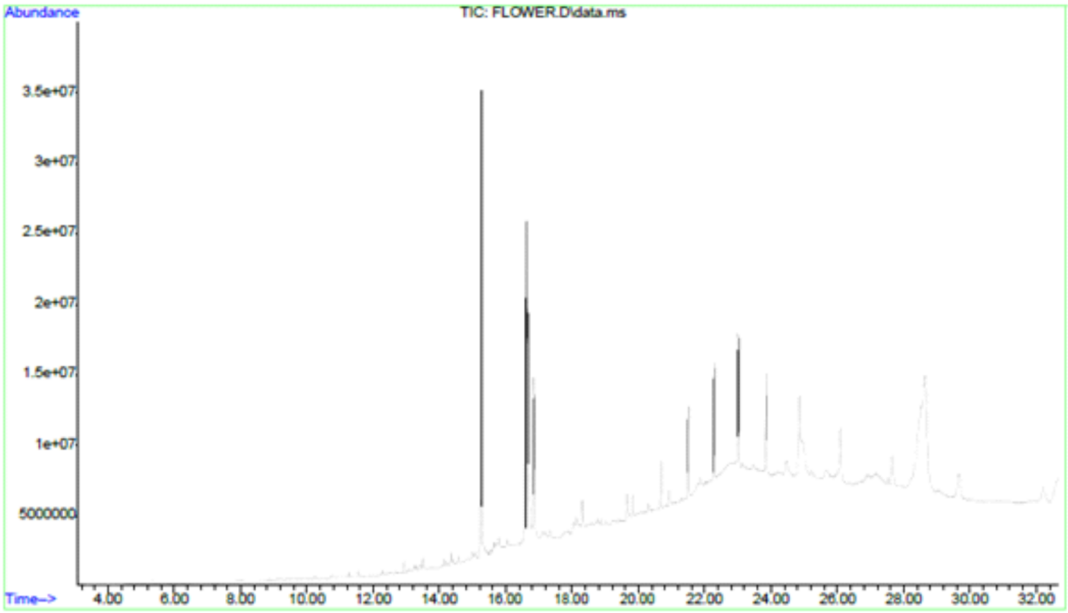

Fig. 3: GC-MS chromatogram of aqueous extract of *C. spicatus* rhizome

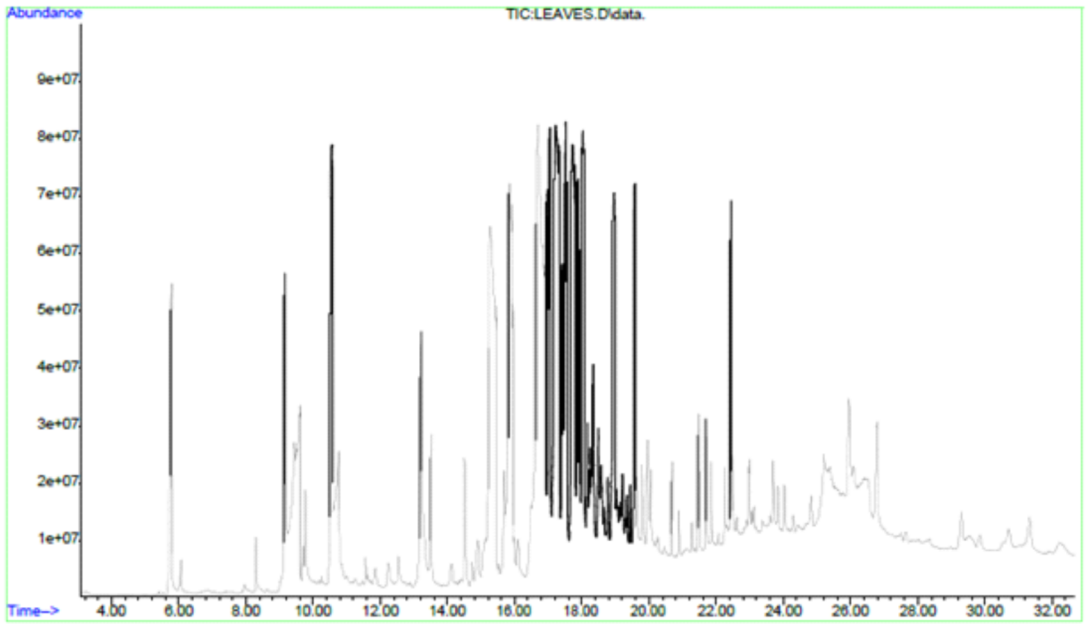

Fig. 4: GC-MS chromatogram of aqueous extract of *C. spicatus* leaves

Conclusion

Based on the phytochemical screening, UV-Spectrophotometry and GC-MS analysis of aqueous extract of *Costus spicatus* leaf and rhizome investigated in this study, the rhizome extract contains more active organic compounds compared to the leaf extract and therefore can be further explored in phytomedicinal researches and drug discovery.

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