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GCMS ANALYSIS AND ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS OF PLANT, *TYPHONIUM DIVARICATUM*

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Abstract
Medicinal plants are of tremendous interest to biotechnology researchers because the majority of drug firms rely on medicinal plants for the production of pharmaceutical compounds. This study extends our understanding of plant chemical composition and provides evidence to support ethno-medicinal use of the plant by investigating antioxidant activity. The present study is aimed to investigate the antioxidant properties using various solvent extracts including ethanol, methanol, chloroform, butanol and aqueous from *Typhonium divaricatum* were quantified using standard methods. The GCMS analysis revealed that, the ethanol, methanol, chloroform, butanol and aqueous extracts of *Typhonium divaricatum* exhibited 1, 14, 5, 1 and 2 compounds respectively. The results of the antioxidant properties of aqueous extracts of *T. divaricatum* revealed the potent free radical quenching properties were 54.86 µg/ml (DPPH); 216.54 µg/ml (Hydroxyl radical) and effective reducing antioxidant power activity with concentration of the extract increased. This study proved that the extract from *T. divaricatum* had the effectiveness for observed antioxidant properties against unsecure free radicals.

Keywords: *antioxidants, Typhonium divaricatum, DPPH, Free radicals, GCMS*

1. Introduction

Traditional medicine based on plant extracts has been shown to be therapeutically useful and less harmful than existing pharmaceuticals. The type of solvent used in the extraction technique has a substantial impact on the successful detection of physiologically active compounds from plant material (Awaad et al., 2011; Lalitha and Jayanthi, 2012). Alcohols (ethanol or methanol), diethyl ether, chloroform, ethyl acetate, n-butanol, and water have all been employed to extract active components from plants (Poulson & Preime, 1998).

The extraction process given is simple, quick, and inexpensive, with minimal solvent consumption. The GC-MS method for extract analysis can be a fascinating tool for measuring the level of some active principles in herbs used in cosmetic, medicines, pharmaceutical, or food industries,

environmental, and forensic applications. It integrates two analytical techniques into a single method for studying chemical compound combinations. The components of the mixture are separated using gas chromatography, and each component is analyzed separately using mass spectroscopy. This approach is particularly useful for detecting biological volatile organic compounds and the volatile profile characteristics associated with them (Zhang et al., 2009). It's also used in areas including medicine, the environment, and chemical engineering.

Antioxidant defense mechanisms, as well as multiple enzymatic and non-enzymatic antioxidant units, are found in living organisms as a safeguard against the accumulation of reactive oxygen species (ROS) (Abdel-hameed, 2009). These antioxidants, according to Isabelle et al. (2010), protect free radicals from damaging DNA, proteins, lipids, and other molecules. They also aid in the scavenging of free radicals and the repair of enzymes involved in cellular growth (Cadenas, 1996). The amount of security provided by an antioxidant chemical is determined by its concentration, antioxidant interaction status, and reactivity toward specific reactive oxygen species. Many researchers have concentrated on natural antioxidants, and many crude extracts and pure natural substances have been discovered to have antioxidant effects in the plant world (Santharam et al., 2015). Antioxidant capabilities have been studied in a broad range of medicinal plants. Natural antioxidants, whether in the form of raw extracts or chemical components, are extremely effective in preventing oxidative stress-related damage (Sayed et al., 2012).

Typhonium divaricatum is found in South East Asia, where it grows in natural, humid environments such as near streams. At maturity, the plant produces flowers that end in a long filament like a rodent's tail. As a result, the common name rodent tuber was coined. The species belongs to the Araceae family and is a type of wild plant used to treat cancer/tumor. Typhonium consists of approximately 40 species that are found throughout tropical and subtropical Asia and extend southward to Australia. *T. divaricatum* is a one-foot-tall green plant. Its blossom has a long filament that resembles a mouse's tail (Aulton et al.,1988). The plant thrives in soft, wet, gloomy conditions. It is now grown in Malaysian and Singaporean households for its therapeutic potential. Because the plant is highly irritating to the skin and mucous membranes, caution should be exercised when preparing it for usage. It is a traditional Chinese medicinal plant with antimicrobial, anti-inflammatory, anti-viral, and anti-cancer effects (Gopukumar et al., 2016; Dineshbabu and Brintha, 2021). The objective of this work was to determine the antioxidant activity of *T. divaricatum* extracts in ethanol, methanol, chloroform, butanol, and aqueous form.

2. Materials and methods

Collection and preparation of plant

In the present study plant *Typhonium divaricatum* was selected based on distribution and the information collected from the literature. The fresh whole plant of *Typhonium divaricatum* is collected. Washed in running tap water and respectively rinsed with distilled water to remove the dirt and dust particles which is present in it. The washed plant was dried using shade dried method and powdered using mortar and pestle.

After the setup of Soxhlet apparatus using clamps and mounts. The round bottom flask is filled with solvent based on the nature of solvent (non-polar to polar = Chloroform > Butanol > Ethanol > Methanol > Aqueous), around 250-300 ml of solvent is filled in the round bottom flask. Cotton is used to cover the hole of siphon or capillary tube in need to avoid the powdered sample to get settled in round bottom flask and also in siphon tube. The powdered sample were rolled in a cotton cloth and placed in the soxhlet thimble. Once the condenser is filled with running tap water. Isomantle (heat source) is used to evaporate the solvent, the temperature was fixed about the heating point of desired solvents. Repeatedly fifteen times the extraction is carried out by running the soxhlet apparatus. The extract was collected and used for further analysis.

GCMS analysis

Plant extracts of *T. divaricatum* were also analyzed for the phytoconstituents present by "GC-MS (Thermo Scientific Co. Trace 1300)". Temperature was set as, initially for 70°C for 2 minutes. hold and then it was increased 7°C/minutes up to 200°C and then again it was accelerated 5°C/minutes. up to 220°C with 5 minutes. hold. 220°C temperature was set as injector temperature. The scanning range of mass was from 35 to 400 (m/z). Data peak processing was analyzed by means of Excalibur (software). Phytoconstituents detected were verified on the basis of their relative retention time and their peak area with the NIST and LIB database in the GC-MS system. The spectrum of the unknown constituents was compared with the spectrum of known constituents stored in the NIST library and also compared with the available literature. The compound name, molecular weight, molecular formula, peak area percentage and their potent biological functions of the all the extracts were ascertained.

***In vitro* antioxidant studies**

DPPH radical scavenging activity

The free radical scavenging activity of the fractions was measured in vitro by 2,2′ - diphenyl-1-picrylhydrazyl (DPPH) assay according to the standard method (Williams et al., 1995). The stock solution was prepared by dissolving 24 mg DPPH with 100 ml of ethanol stored at 20°C until required. The working solution was obtained by diluting DPPH solution with ethanol and 3 ml aliquot of this solution was mixed with 1 ml of sample at various concentrations (100, 200 and 300 µg/ml). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The control was prepared without any sample and scavenging activity was estimated based on the percentage of DPPH radical scavenging as the following equation.

$$Percentage\ of\ inhibition = \left[\frac{(control\ OD - sample\ OD)}{(control\ OD)}\right] \times 100$$

Hydroxyl Radical Scavenging Activity

The reaction mixture contained 0.8 ml of phosphate buffer solution (50 mmol L^{-1}, pH 7.4), 0.2 ml of a sample of different concentrations (20, 40, 60, 80 and 100 µg/ml), 0.2 ml of EDTA (1.04 mmol L^{-1}), 0.2 ml of $FeCl_3$ (1 mmol L^{-1}), and 0.2 ml of 2-deoxyribose (60 mmol L^{-1}). The mixtures were kept in a water bath at 37°C and the reaction was started by adding 0.2 ml of ascorbic acid (2 mmol L^{-1}) and 0.2 ml of H_2O_2 (10 mmol L^{-1}). After incubation at 37°C for 1 hour, 2 ml of cold thiobarbituric acid (10 g L^{-1}) was added to the reaction mixture followed by 2 ml of HCl (25%). The mixture was heated at 100°C for 15 minutes and then cooled down with water. The absorbance of solution was measured at 532 nm with a spectrophotometer. The hydroxyl radical scavenging capacity was evaluated with the inhibition percentage of 2-deoxyribose oxidation on hydroxyl radicals (Halliwell and Arnoma, 1987).

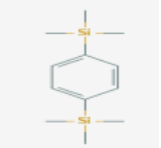
The scavenging percentage was calculated according to the following formula:

$$Scavenging\ effect\ (\%) = \left[\frac{(control\ OD - sample\ OD)}{(control\ OD)}\right] \times 100$$

3. Results

The GCMS chromatogram of various extracts of *T. divaricatum* is presented in Figure 1 to 5 below. The analysis of the active ingredients in the *T. divaricatum* indicated the presence of chemical compounds that could contribute towards the medicinal properties of the plant (Table 1 to 5). The GC-MS results showed that ethanol extracts of *T. divaricatum* has one bioactive compound (Table 1, Fig. 1) such as 1,4- Bis (trimethylsi lyl) benzene with 100% of area covered.

Table 1. Active compounds in the ethanol extracts of *T. divaricatum*

Sl. No.	Name	Retention time (mins)	Mol. formula	Mol. Wt. (g/mol)	Area (%)	Structure	Biological properties
1	1,4-Bis(trimethylsilyl)benzene	16.780	$C_{12}H_{22}Si_2$	222.47	100		As a precursor for developing silicon carbide coating using plasma-assisted chemical vapor deposition (CVD) process as a precursor for developing silicon carbide coating using plasma-assisted chemical vapor deposition (CVD) process (Wikipedia)

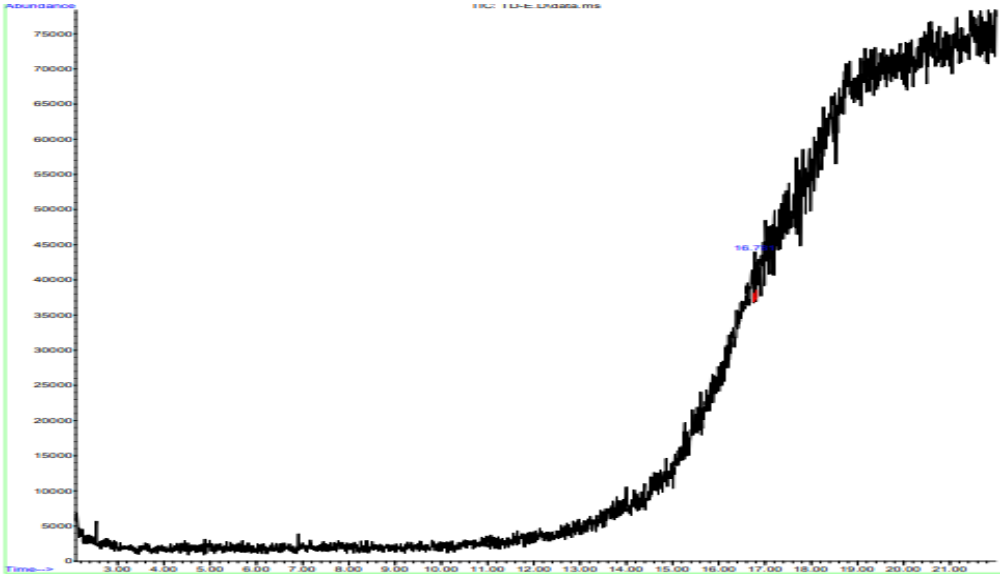
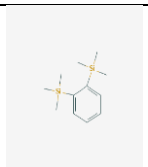
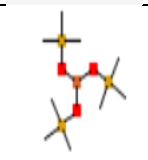
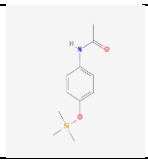
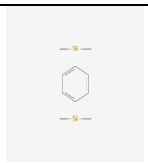
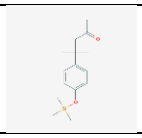
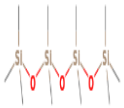
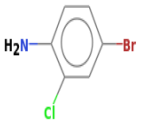
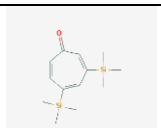
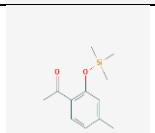
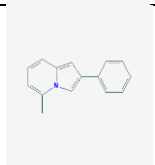
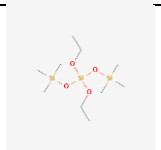
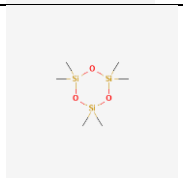
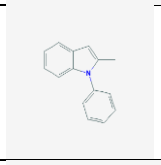
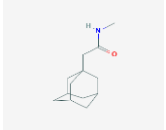


Figure 1. GCMS chromatogram of ethanol extract of *T. divaricatum*

GC-MS chromatogram of methanol extract of *T. divaricatum* showed appreciable amount of phyto constituents with notable area % viz., 2-Methyl-7-phenylindole (19.42%), Trimethyl[4-(2-methyl-4-oxo-2-peentyl) phenoxy]silane (12.74%), 2,4,6-Cycloheptatrien-1-one,3,5 (11.24%), Cyclotrisiloxane, hexamethyl- (11.21%), 1,4-Bis (trimethylsilyl)benzene (6.88%), Arsenous acid, tris(trimethylsil (6.87%), 1,2,4-Benzenetricarboxylic acid (6.56%), Tetrasiloxane, decamethyl- (5.77%), 4-Methyl-2-trimethylsilyloxy-acetophenon (5.09%), 1,2-Bis(trimethylsilyl) benzene (4.04%), Silicic acid, diethylbis (trimethylsilyl) silicate (3.98%), Benzenamine,4-bromo-2-chloro- (3.48%), Acetamide,N-[4-(trimethylsilyl) (2.17%) and 5-Methyl-2-phenylindolizine (1.96%) (Table 2; Fig. 2).

Table 2. Active compounds in the methanol extracts of *T. divaricatum*

Sl. No.	Name	Retention time (mins)	Mol. formula	Mol. Wt. (g/mol)	Area (%)	Structure	Biological properties
1	1,2-Bis(trimethylsilyl) benzene	16.450	$C_{12}H_{22}Si_2$	222.47	4.04		benzyne precursors and certain luminescent π-conjugated materials (Lorbach et al., 2010)
2	Arsenous acid, tris(trimethylsil	16.620	C9H27AsO3Si3	342.49	6.87		-
3	Acetamide,N-[4-(trimethylsilyl)	16.677	$C_{11}H_{17}NO_2Si$	223.34	2.17		used in analytical chemistry to increase the volatility of analytes (Wikipedia)

4	1,4-Bis (trimethylsilyl)benzene	16.781	$C_{12}H_{22}Si_2$	222.47	6.88		As a precursor for developing silicon carbide coating using plasma-assisted chemical vapor deposition (CVD) processAs a precursor for developing silicon carbide coating using plasma-assisted chemical vapor deposition (CVD) process (Wikipedia)
5	Trimethyl[4-(2-methyl-4-oxo-2-peentyl) phenoxy]silane	17.093	$C_{15}H_{24}O_2Si$	264.43	12.74		-
6	Tetrasiloxane, decamethyl-	17.168	$C_{10}H_{30}O_3Si_4$	310.68	5.77		anti-foaming, skin protectants and skin conditioning agents (IMAP Single Assessment Report)
7	Benzenamine,4-bromo-2-chloro-	17.310	C_6H_5BrClN	206.46	3.48		treatment of Hyperoxaluria and related diseases (Bai et al., 2020)
8	2,4,6-Cycloheptatrien-1-one,3,5...	17.442	$C_{13}H_{22}OSi_2$	250.48	11.24		-
9	4-Methyl-2-trimethylsilyloxy-acetophenon	17.773	$C_{12}H_{18}O_2Si$	222.35	5.09		-
10	5-Methyl-2-phenylindolizine	17.858	$C_{15}H_{13}N$	207.27	1.96		-
11	Silicic acid, diethylbis (trimethylsilyl) silicate	19.144	$C_{10}H_{28}O_4Si_3$	296.58	3.98		-
12	Cyclotrisiloxane, hexamethyl-	19.192	$C_6H_{18}O_3Si_3$	222.46	11.21		antimicrobial and antioxidant (Ismail et al. 2020)
13	2-Methyl-7-phenylindole	19.456	C15H13N	207.27	19.42		-
14	1,2,4-Benzenetricarboxylic acid	20.553	$C_9H_6O_6$	210.14	6.56		adhesion promoter and demineralizing monomer (Sina, 2011)

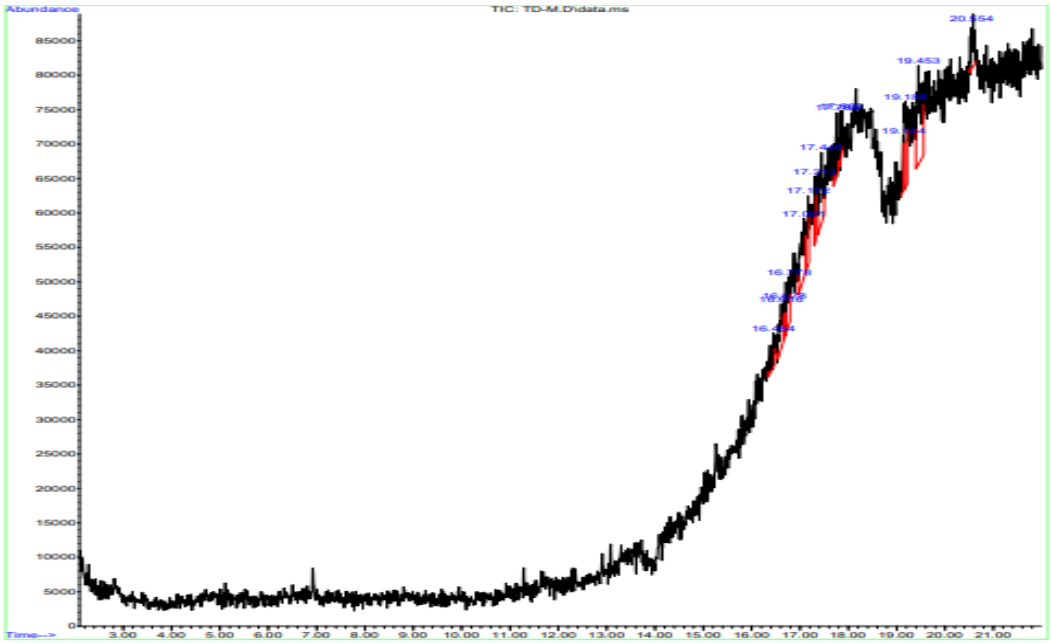
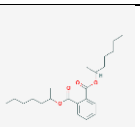
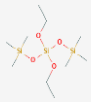


Figure 2. GCMS chromatogram of methanol extract of *T. divaricatum*

Table 3 and Fig. 3 display the target mass ions (m/z) and retention times of all recognised compounds in *T. divaricatum*. The results showed that, chloroform extracts of *C. bicolor* contained 5 bioactive compounds of Phthalic acid,di (hept-2-yl)ester, Silicic acid, diethylbis(trimet, Benzene, 2-[(tert-butyldimethyls, Arsenous acid,tris(trimethylsil and 1,2-Bis(trimethylsilyl)benzene. The primary component of chloroform extracts of *T. divaricatum* was Propranolol (50.02%).

Table 3. Active compounds in the chloroform extracts of *T. divaricatum*

Sl. No.	Name	Retention time (mins)	Mol. formula	Mol. Wt. (g/mol)	Area (%)	Structure	Biological properties
1	Phthalic acid,di (hept-2-yl)ester	16.733	C22H34O4	362.5	32.05		anti-inflammatory, antimicrobial properties (Osuntokun and Ogunleye, 2017)
2	Silicic acid, diethylbis(trimet...	19.598	C10H28O4Si3	296.58	50.02		-
3	Benzene, 2-[(tert-butyldimethyls..	19.712	$C_{16}H_{28}OSi$	264.48	4.84		-
4	Arsenous acid,tris(trimethylsil	19.806	$C_9H_{27}AsO_3Si_3$	342.49	7.28		used as *a herbicide, pesticide (*Wikipedia*)*
5	1,2-Bis(trimethylsilyl)benzene	21.470	$C_{12}H_{22}Si_2$	222.47	5.28		benzyne precursors and certain luminescent π-conjugated materials (Lorbach et al., 2010)

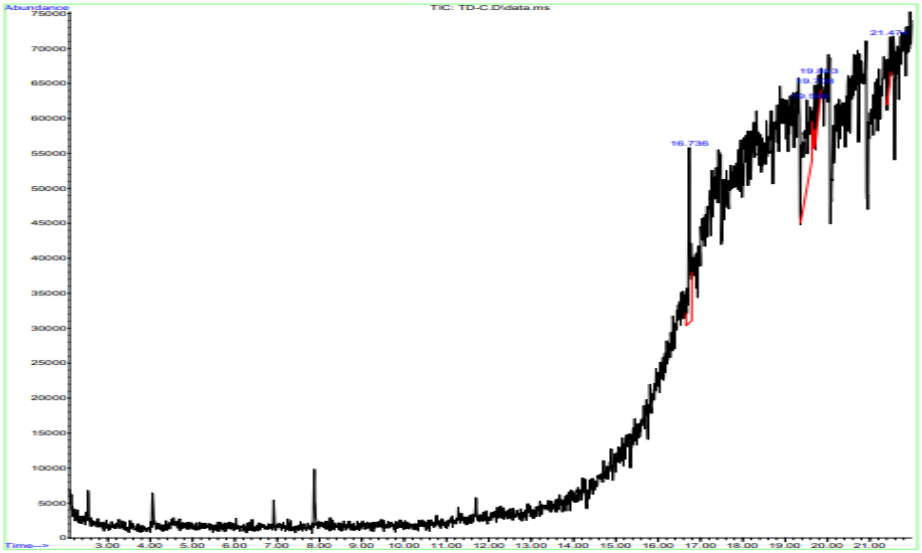
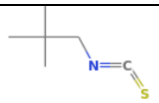


Figure 3. GCMS chromatogram of chloroform extract of *T. divaricatum*

The GC-MS results showed that butanol extracts of *T. divaricatum* has one bioactive compound (Table 4, Fig. 4) such as Neopentyl isothiocyanate with 100% of area covered.

Table 4. Active compounds in the butanol extracts of *T. divaricatum*

Sl. No.	Name	Retention time (mins)	Mol. formula	Mol. Wt. (g/mol)	Area (%)	Structure	Biological properties
1	Neopentyl isothiocyanate	4.054	$C_6H_{11}NS$	129.223	100		-

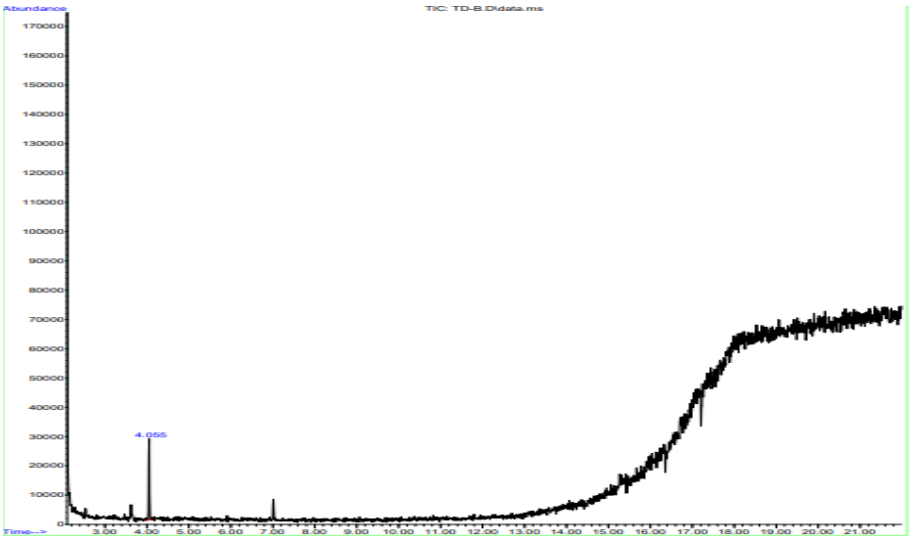
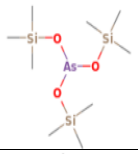
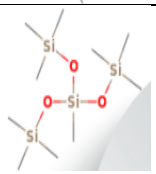


Figure 4. GCMS chromatogram of butanol extract of *T. divaricatum*

The key compounds identified in the aqueous extract of *T. divaricatum* viz Arsenous acid, tris (trimethylsil. (89.68%), and Methyltris(trim ethylsiloxy) silane (10.32%) (Table 5; Fig. 5).

Table 5. Active compounds in the aqueous extracts of *T. divaricatum*

Sl. No.	Name	Retention time (mins)	Mol. formula	Mol. Wt. (g/mol)	Area (%)	Structure	Biological properties
1	Arsenous acid, tris (trimethylsil.	19.286	$C_9H_{27}AsO_3Si_3$	342.48	89.68		used as *a herbicide, pesticide (*Wikipedia*)*
2	Methyltris(trim ethylsiloxy) silane	19.371	$C_{10}H_{30}O_3Si_4$	310.68	10.32		used in the study of thermal rearrangement of branched-chain methylpolysiloxanes (Toronto Research Chemicals)

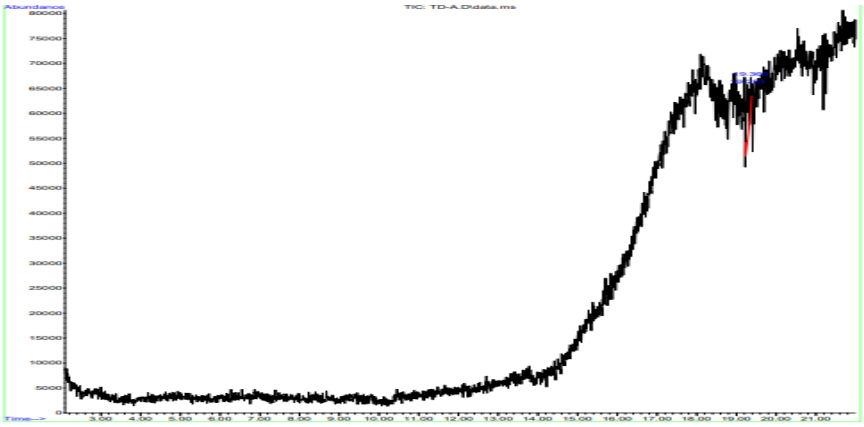


Figure 5. GCMS chromatogram of aqueous extract of *T. divaricatum*

Antioxidant activity

The ethanol, methanol, chloroform, butanol and aqueous extracts of the *T. divaricatum* samples were tested for radical scavenging activities using DPPH, and hydroxy radicals. All the tested extracts in the present study exhibited concentration dependent scavenging activity on hydroxyl radical and DPPH radical and the data on the scavenging activity of the extracts are presented in Fig. 6 and 7.

DPPH radical scavenging activity

Aqueous extracts of *T. divaricatum* showed maximum scavenging activity on DPPH when compared to the other solvent extracts tested, whereas the standard, Ascorbic acid showed better performance on the DPPH free radical inhibition (Fig. 6).

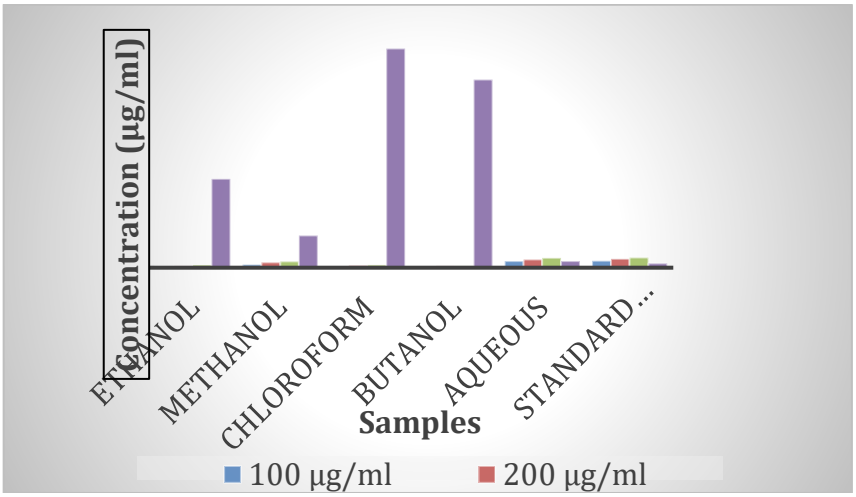


Figure 6. DPPH radical scavenging activity of different extracts of *T. divaricatum* plant at various concentration

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity was quantified by measuring the inhibition of the degradation of 2- deoxyribose by the free radicals generated by the Fenton reaction. The hydroxyl radical scavenging effect of different extracts of *T. divaricatum* was compared with the same doses of ascorbic acid ranging from 100 - 300 µg/ml. The IC_{50} values in hydroxyl scavenging activities were in the order of aqueous > methanol > ethanol > chloroform > butanol (Fig. 7). When compared to ascorbic acid; the hydroxyl scavenging activity of the extract was found to be high.

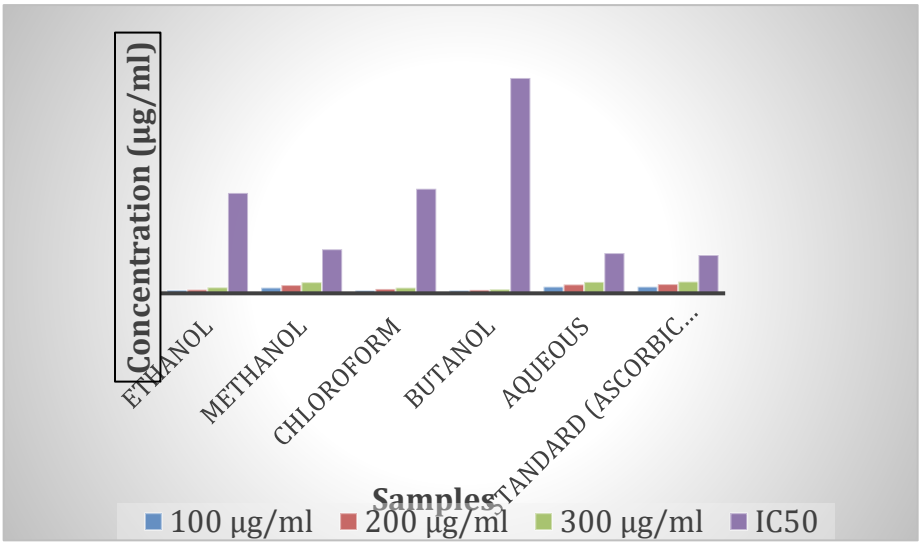


Figure 7. Hydroxyl radical scavenging activity of different extracts of *T. divaricatum* plant at various concentration

4. Discussion

One of the first steps in determining the type of active principles in medicinal plants and whether a certain compound or group of compounds are present in a plant species is to conduct a GCMS study. The key components' presence was confirmed by the GC-MS spectrum profile and their retention times. The peak heights display the relative concentrations of the various elements found in the extracts. The phytoconstituents were characterised and identified by comparing the mass spectra of the constituent with the NIST library.

Some of the *T. divaricatum*'s discovered phytochemicals, such as Tetrasiloxane, decamethyl, a non-cyclic silicone oligomer, are employed in the methylation of mercury (II) salts. According to the IMAP Single Assessment Report, it is changed by a particular microflora and functions as an anti-foaming, skin-protective, and skin-conditioning agent. Benzenamine,4-bromo-2-chloro- is used in preparation of 1H-1,2,3-Triazole-5-carboxylic Acid derivatives as Glycolate Oxidase inhibitors for treatment of Hyperoxaluria and related diseases (Bai et al., 2020). Cyclotrisiloxane, hexamethyl has antimicrobial and antioxidant properties (Ismail et al. 2020), Common plasticizers used to increase the flexibility and workability of polymeric materials are phthalic acid and di (hept-2-yl) ester. Due to their distinctive qualities, such as good insulation, high strength, excellent corrosion resistance, low cost, ease of fabrication, and possessing anti-inflammatory and antimicrobial properties, PAEs have been widely used in a variety of consumer products, including cosmetics, food packaging, building materials, medical supplies, home furnishings, etc. (Osuntokun and Ogunleye, 2017). Arsenic tris(trimethylsilyl) acid functions as a herbicide and has a total of 42 bonds, including 15 non-H bonds and 6 rotatable bonds.

The importance of natural antioxidants and their health advantages has increased recently. Many complex diseases are prevented and treated with medication formulations based on antioxidants. Natural antioxidants are mostly found in plants, which also create a variety of secondary metabolites with antioxidative properties and potential medicinal applications. The most prevalent antioxidants in raw plant material are polyphenols. Their redox characteristics, which promote their action as

reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators, and ferritin reductants, are the foundation of their antioxidant activity. The presence of reductants, which break the free radical chain by donating a hydrogen atom or inhibit the development of peroxide, is typically related with the reducing ability (Kumaran and Karunakaran, 2006). We investigate the antioxidant properties of the plant *T. divaricatum or* after the GCMS analysis revealed that a number of compounds have these properties.

The study's evaluation of the plants *T. divaricatum* revealed that their high antioxidant potential and diversity in antioxidant properties. With an IC_{50} value of 54.86 µg/ml, the aqueous extracts of plant sample from the *T. divaricatum* were able to scavenge DPPH radical activity. According to Nunes et al. (2012), 2,20-diphenyl-1-picrylhydrazyl radical (DPPH) solution bleaching can be used to assess the potential of organic substances to donate electrons. The technique relies on scavenging DPPH by including an antioxidant or radical species that causes the DPPH solution to become less coloured. The concentration and efficacy of the antioxidants are inversely correlated with the degree of colour change. Considerable free radical scavenging activity of the tested chemical is shown by a considerable drop in the absorbance of the reaction mixture (Krishnaiah et al., 2011). These results from the current investigation suggested that the decolorization of the stable DPPH radical solution was caused by the medicinal plant samples evaluated having relatively stronger antioxidant capacity. *T. divaricatum* extracts in aqueous shown strong inhibitory efficacy in DPPH. According to several research (Pensec et al., 2016), the relationship between antioxidant activity and phenolic content of plant extracts is linear. The study's findings indicate that the plant extract contains phytochemical elements that can donate hydrogen to a free radical in order to scavenge the potential harm.

One of the powerful reactive oxygen species in the biological system is the hydroxyl radical. It damages cells by reacting with their polyunsaturated fatty acid moieties in the cell membrane phospholipids (Sofowara, 1993). The hydroxyl radical is viewed as a harmful species in pathophysiological processes since it can harm practically every biological system molecule and has a role in the development of cancer, mutagenesis, and cytotoxicity. The reaction between H_2O_2 and the ferrous that would react with 2-deoxyribose resulted in the production of hydroxyl radicals. The addition of TBA reagent, which would become red if malonaldehyde were to be produced as a result of the reaction between the radical and 2-deoxyribose, stopped the process. The ability of an extract to scavenge hydroxyl radicals is closely correlated with its antioxidant activity, which is shown by the extract's low intensity of red colour (Gulcin et al., 2005). When added to the reaction mixture, the extracts of *T. divaricatum* efficiently scavenged the hydroxyl radicals and stopped the degradation of 2-deoxyribose. The significantly stronger antioxidant response of the *T. divaricatum* extracts in the current study when compared to ascorbic acid may be useful in identifying the main sources of the natural antioxidant reaction. These findings imply that the plant extract from *T. divaricatum* is a more effective hydroxyl radical scavenger than ascorbic acid, based on the IC_{50} values of all the extracts.

5. Conclusion

Medicinal plants, which are the basis of traditional medicine, have been the subject of intense pharmacological research in recent decades, owing to the recognition of the value of medicinal plants as potential sources of new compounds with therapeutic value and as sources of lead compounds in drug development. Thus, GC-MS analysis was used to identify bioactive chemicals in T. divaricatum, which revealed the existence of 23 compounds. *T. divaricatum* is thought to be an effective antioxidant agent that can protect humans from oxidative stress-induced illnesses. Because of the existence of these phytochemicals and bioactive substances, this study concludes that *T. divaricatum* may serve as a new potential source of medications.

6. References

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