International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

# Gc-MS Analysis and In-vitro Antioxidant Activities of Cissus Quandrangularis Stem Extracts

**Sruthi A[1], Dr. Karpagapandi. L[2]**

[1]Department of Food science and Technology, Palar Agricultural College, Ambur – 635805
[2]Department of Family Resource Management, Tamil Nadu Agricultural University, Madurai - 625 104

**ABSTRACT:**

Plants with therapeutic constituents are gaining prominence in the fields of food and pharmacological industries. Nowadays, researches are focussed mainly on drugs from natural sources with minimal side effects. The aim of the present study was to investigate the phytochemical constituents and antioxidant activity of *Cissus quadrangularis* stem in Methanol extract. The phytochemical constituents were identified by Gas Chromatography-Mass Spectrometry (GC-MS) analytical method and *in-vitro* antioxidant activity of methanol extract was investigated by DPPH (2, 2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity. The total antioxidant activity revealed that fresh stem has more antioxidant activity compared to dried powdered stem. The Phytochemical analysis carried out by GC-MS technique indicated that thirty two compounds were present in *Cissus quadrangularis* stem extract. Thus the results anticipated the therapeutic potency of the plant and its basis for further phytochemical and nutraceutical investigation.

**Keywords: *Cissus quadrangularis*, stem, GC-MS, antioxidant activity, DPPH assay**

## INTRODUCTION:

The medicinal plants are used from centuries for healing and curing of human ailments since it contains phytochemical constituents. The identification and isolation of such active compounds makes it efficacious in therapeutic applications. The use of medicinal plants as a source of medicine has been inherited and is an important component of the health care system. Plant extracts and bioactive compounds extricated from medicinal plants are utilized as a contender for antibacterial, antifungal and antiviral therapy (Hussain *et al.* 2013). Moreover, a quarter of the allopathic medications are based on compounds isolated from natural products. With increase in drug recalls resulting from severe side effects, the pharmaceutical industry is interested in finding newer drugs from natural sources with fewer or no side-effects. Recently, these traditional medicines are receiving more scientific support which helps in not only authenticating the use of these medicines for treatment but also understanding the mechanism of action of these drugs (Fernandes *et al.* 2012).

*Cissus quadrangularis* is the most prevalent species, belonging to the family Vitaceae, commonly known as Pirandai, Hadjod, Veldt Grape, Devil's backbone, Asthisamharaka and bone healer. *Cissus quadrangularis* is an ancient medicinal plant and an active ingredient of one Ayurvedic formula called *Laksha Gogglu*, for healing bone related disorders. Its stem is used for food preparation

in India. Traditionally it is used to medicate various diseases such as gastritis, asthma, anaemia, indigestion, eye and ear diseases, irregular menstruation, skin diseases, piles, gastric ulcer, constipations, fractured bones, burns and wounds. It is a multipurpose plant used traditionally for different healing treatments (Bhujade *et al.* 2012 and Manimekalai *et al.* 2015). Earlier works on *Cissus quadrangularis* reported its effectiveness on the management of obesity and complications associated with metabolic syndrome as well as its antioxidant and free radical scavenging activity *in-vitro* (Mohanambal *et al.* 2011). *Cissus quadrangularis* has potent fracture healing property and antimicrobial, antiulcer, antioxidative, antiosteoporotic, gastroprotective, cholinergic activity as well as beneficial effects on cardiovascular diseases (Manimekalai *et al.* 2015). *Cissus quadrangularis* has been recognized as a novel source of carotenoids, triterpenoids and ascorbic acid and is manifested to have potential for medical effects, including Gastro protective activity, lipid metabolism and oxidative stress. The uses and various therapeutic activities have made the plant a valuable medicinal herb (Ghouse *et al.* 2015). Panthong *et al.* (2007) revealed that anti-inflammatory effect of crude extract from *Cissus quadrangularis*. Its anti-inflammatory effect could be produced by the flavonoids especially luteolin, and by β-sitosterol. Panthong *et al.* (2007) stated that *Cissus quadrangularis* also possesses analgesic effect, which can be very useful in painful haemorrhoid. The study proved the traditional use of *Cissus quadrangularis* as an anti haemorrhoidal drug in Thai folk medicine. A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in nutraceutical, drug and therapeutical foods research. The quantitative determination and some physiochemical and phytochemical parameters are useful for setting standards for therapeutic applications.

## MATERIALS AND METHODS

### *In-vitro* Antioxidant Assay

#### *Sample preparation*

Known quantities of fresh and dried samples (2g) were taken and 15ml of 80% methanol was added and kept at room temperature for 30 minutes. The three supernatants was pooled, centrifuged at 6000 rpm for 15 min and filtered through Whatman No.1 filter paper.

#### *Antioxidant activity by DPPH RSA (In-vitro model)*

Total antioxidant capacity was determined by DPPH (2, 2- Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity (Brand –Williams et al. 1995 and Tadhani *et al.* 2009). Sample (0.3, 0.5, 0.7ml) taken and made upto 1ml with methanol, Standard (0.1,0.2,0.3,0.4 ml) was taken and made up to 1ml with methanol, Blank (1ml methanol) were taken. 3ml of DPPH reagent was added and incubated for 20 min at 37°C and absorbance was read at 517 nm.

$$\%\ \text{inhibition of sample} = (\text{Control (OD)-Sample (OD)}) / \text{Control(OD)} \times 100$$

### Phytochemical analysis by GC-MS

#### *Sample preparation*

The plant was cleaned and dried in cabinet drier at 40°C and it was finely powdered. The powdered sample was stored in air tight container and used for GC-MS analysis. 10g of powder was taken and saturated in 100ml of HPLC graded Methanol. It was left for 24 hrs by frequent shaking of sample. It was initially filtered with muslin cloth and then with Whatman No.1 filter paper. The filtered extract was then concentrated in Flash Evaporator after which it was filtered with anhydrous sodium

sulphate to get water free extract. The water free extract was used for analysing bioactive components by GC-MS.

***GC-MS analysis***

GC-MS analysis was carried out on Shimadzu GC-MS QP 2020 system comprising auto sampler and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument employing following conditions: Column Elite-1 fused silica capillary column (30mm×0.25mm I.D ×1 µ M df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 1.0µl was employed (split less) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time is 34 minutes.

## RESULT AND DISCUSSION

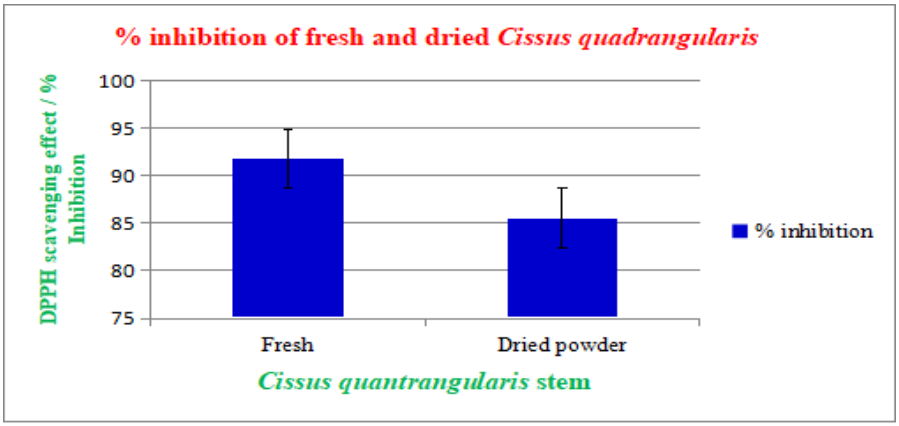
***Total Antioxidant activity of Cissus quadrangularis by DPPH Radical Scavenging Activity***

The free radical scavenging potential of fresh and dried *Cissus quadrangularis* stem extract was tested by the DPPH (*in-vitro* model). Antioxidants react with DPPH, which is a stable free radical and converts to 1, 1-diphenyl, 2-picrylhydrazine. The antioxidant activity of methanolic extract of *Cissus quadrangularis* fresh stem was compared with dried stem. It has revealed that fresh stem has more antioxidant activity compared to dry stem. Percent (%) inhibition of fresh sample is 91.78 whereas % inhibition of dried sample is 85.5. Devika (2019) also reported that antioxidant activity of ethanolic extract of *Cissus quadrangularis* was found to be 51.17% at 300 µg/mL concentration. Dhanasekaran (2020) revealed that methanolic extract of *Cissus quadrangularis* exhibited potent antioxidant property that can be used for treating tumorigenesis. Murthy *et al.* (2003) concluded that *Cissus quadrangularis* can be used as an antibacterial agent and as an antioxidant in several applications.

**Table 1. *In-vitro* Total antioxidant activity of *Cissus quadrangularis* by DPPH**

| S.No. | *Cissus quadrangularis* stem | DPPH free radical inhibition (%) |
|---|---|---|
| 1. | Fresh | 91.78 ± 6.47 |
| 2. | Dried powder | 85.50 ± 5.99 |

**Figure 1. *In-vitro* Total antioxidant activity of *Cissus quadrangularis* by DPPH**



***Identification of phytochemicals in Cissus quadrangularis by GC-MS:***

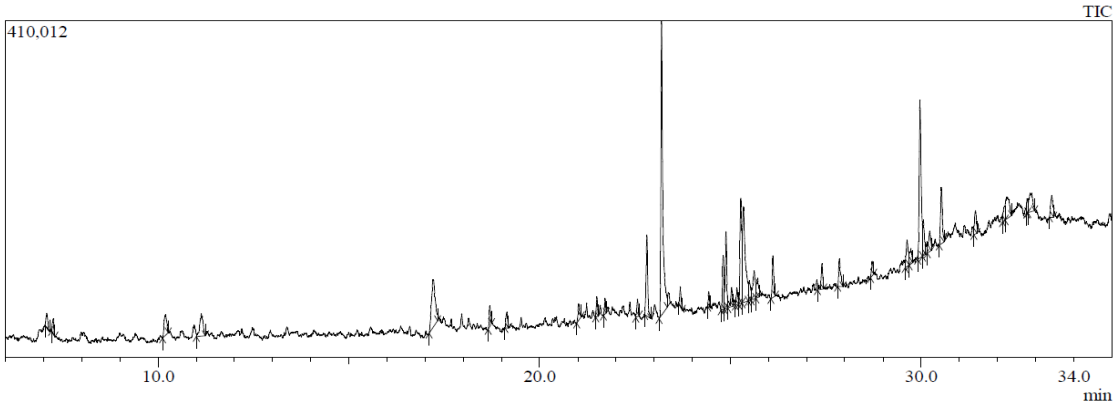
Gas Chromatography-Mass Spectrometric spectrum of the methanol extract of *Cissus quadrangularis* stem showed 32 peaks (Fig 2.) indicating the presence of thirty two bio-active compounds. Chemical compounds indentified with their molecular formula, molecular weight, retention time and % of peak area were presented in the Table 2.

**Table 2. Identified phytochemical compounds in methanol extract of *Cissus quadrangularis* stem**

| S. No | Name of the Compounds | Retention Time | Molecular Formula | Molecular Weight | % Peak Area |
|---|---|---|---|---|---|
| 1. | 3,3-Dimethoxy-2-butanone | 7.084 | $C_6H_{12}O_3$ | 132 | 0.77 |
| 2. | 1,3-Dioxolane-4-methanol, 2-ethyl- | 7.250 | $C_6H_{12}O_3$ | 132 | 0.63 |
| 3. | Decane | 10.190 | $C_{10}H_{22}$ | 142 | 1.77 |
| 4. | 1-Butanamine, 3-methyl-N-(3-methylbutylide | 11.135 | $C_{10}H_{21}N$ | 155 | 2.36 |
| 5. | 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro- | 17.220 | $C_4H_9NO_5$ | 151 | 5.61 |
| 6. | Dodecanoic acid | 18.696 | $C_{12}H_{24}O_2$ | 200 | 0.86 |
| 7. | 1-(p-Ethoxycarbonyl phenyl)-3-(p-bromophen | 19.140 | $C_{15}H_{14}BrN_3O_2$ | 347 | 0.83 |
| 8. | Tetradecanoic acid | 21.021 | $C_{14}H_{28}O_2$ | 228 | 0.94 |
| 9. | 11-Methyltricosane | 21.505 | $C_{24}H_{50}$ | 338 | 0.76 |
| 10. | 2(1H)-Naphthalenone, 3,4,4a,5,6,7,8,8a.alpha | 21.721 | $C_{15}H_{24}O_3$ | 252 | 0.55 |
| 11. | Hexadecanoic acid, methyl ester | 22.814 | $C_{17}H_{34}O_2$ | 270 | 4.16 |
| 12. | n-Hexadecanoic acid | 23.197 | $C_{17}H_{34}O_2$ | 270 | 17.43 |
| 13. | Eicosane | 23.687 | $C_{20}H_{42}$ | 282 | 0.80 |
| 14. | Cyclooctasiloxane, hexadecamethyl- | 24.434 | $C_{16}H_{48}O_8Si_8$ | 592 | 0.58 |
| 15. | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 24.812 | $C_{19}H_{34}O_2$ | 294 | 2.46 |
| 16. | 7-Hexadecenal, (Z) | 24.887 | $C_{16}H_{300}$ | 238 | 3.73 |
| 17. | Phytol | 25.032 | $C_{20}H_{400}$ | 296 | 1.12 |
| 18. | Methyl stearate | 25.172 | $C_{19}H_{38}O_2$ | 298 | 0.78 |
| 19. | 9,12-Octadecadienoic acid (Z,Z)- | 25.274 | $C_{18}H_{32}O_2$ | 280 | 5.57 |
| 20. | Dichloroacetic acid, tridec-2-ynyl ester | 25.341 | $C_{15}H_{24}Cl_2O_2$ | 306 | 8.75 |
| 21. | 3H-Indazol-3-one, 1,2-dihydro-2-phenyl- | 25.500 | $C_{13}H_{10}N_{20}$ | 210 | 1.27 |
| 22. | p-Methoxycinnamic acid, | 25.715 | $C_{20}H_{26}O_3$ | 314 | 1.35 |

| | | | | | |
|---|---|---|---|---|---|
| | geranyl ester | | | | |
| 23. | Eicosyl isopropyl ether | 26.116 | $C_{23}H_{48}O$ | 340 | 1.87 |
| 24. | Tetracosane | 27.401 | $C_{24}H_{50}$ | 338 | 1.43 |
| 25. | Cyclononasiloxane, octadecamethyl | 29.630 | $C_{18}H_{54}O_9Si_9$ | 668 | 1.80 |
| 26. | 1-Hexacosanol | 29.973 | $C_{26}H_{54}O$ | 382 | 9.64 |
| 27. | Tetrapentacontane | 30.060 | $C_{54}H_{110}$ | 758 | 1.93 |
| 28. | Hexadecanoic acid, 2-hydroxy-1-(hydroxymeth) | 30.225 | $C_{19}H_{38}O_4$ | 330 | 1.37 |
| 29. | Diisooctyl phthalate | 30.533 | $C_{24}H_{38}O_4$ | 390 | 3.35 |
| 30. | Octyltrichlorosilane | 32.175 | $C_8H_{17}Cl_3Si$ | 246 | 0.79 |
| 31. | Tetracosyl acetate | 32.237 | $C_{26}H_{52}O_2$ | 396 | 2.66 |
| 32. | 2H-3,9a-Methano-1-benzoxepin, octahydro-2,2 | 32.778 | $C_{15}H_{26}O$ | 222 | 0.44 |

**Figure 2. Gas Chromatography-Mass Spectrometric spectrum of methanol extract of *Cissus quadrangularis* stem**




It was found that the main constituents of methanol extract of *Cissus quadrangularis* stem 1-Butanamine, 3-methyl-N-(3-methylbutylide (2.36 %), 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro- (5.61 %), Hexadecanoic acid, methyl ester (4.16 %), n-Hexadecanoic acid (17.43 %), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (2.46 %), 7-Hexadecenal, (Z) (3.73 %), 9,12-Octadecadienoic acid (Z,Z)- (5.57 %), Dichloroacetic acid, tridec-2-ynyl ester (8.75 %), 1-Hexacosanol (9.64 %), Diisooctyl phthalate (3.35 %) and Tetracosyl acetate (2.66 %).

The n-Hexadecanoic acid also known as Methyl palmitate, is an anti inflammatory compound which has a molecular formula of $C_{17}H_{34}O_2$. The n-Hexadecanoic acid-, methyl/ethyl ester of hexadecanoic acids are considered as fatty acids and these play important role in biological process (Aleryani *et al.*, 2005 and Bao *et al.*, 2002). Aparna *et al.* (2012) stated that n-hexadecanoic acid which was used for the preparation of medicated oils was used for the treatment of rheumatic symptoms in the traditional medical system of India, Ayurveda.

The 1,3-Propanediol, 2-(hydroxymethyl) -2-nitro- also known as Tris (hydroxymethyl) nitromethane has molecular formula of $C_4H_9NO_5$. Tris (hydroxymethyl) nitromethane products are used in commercial/industrial water-treatment systems and in the oil and gas industry to control bacteria and slime and in the formulation of industrial biocides and preservatives (Anon, 2014).

The 9, 12-Octadecadienoic acid (Z, Z) - has molecular formula of $C_{18}H_{32}O_2$. It is a doubly unsaturated fatty acid, occurring widely in plant glycosides. It is an essential fatty acid in mammalian nutrition and is used in the biosynthesis of prostaglandins and cell membranes (Anon, 2017).

Phytol in the methanol fractions is a diterpene alcohol which functions as a precursor for Vitamins E and K1 and an antioxidant and a preventive agent against epoxide-induced breast cancer carcinogenesis. It's also an effective vaccine adjuvant with no adverse auto-immune effects (Daniet *et al.* 2011).

The Dichloroacetic acid, tridec-2-ynyl ester has molecular formula of $C_{15}H_{24}Cl_2O_2$. It has an anti-cancer property which slows down the tumour in animal studies. The 1-Hexacosanol also known as Ceryl alcohol and Cerotyl alcohol has molecular formula of $C_{26}H_{54}O$. It has shown to exert neurotrophic properties on central neurons and to stimulate phagocytosis in macrophages (Damge *et al.* 1995). 1-Hexacosanol reverses diabetic induced muscarinic hyper-contractility of ileum in rat (Shinbori *et al.* 2006).

## Conclusion

The antioxidant activity and phytochemical parameters are useful for setting standards for therapeutic applications. A wide variety of chemical constituents have been identified from *Cissus quadrangularis* extracts with few cases having specific physiological effects related to identifiable constituents. Therefore, better standardization of extracts is required. The total antioxidant activity of methanolic extract of *Cissus quadrangularis* fresh stem has more antioxidant activity compared to dried powdered stem (% inhibition of fresh sample is 91.78 whereas % inhibition of dried sample is 85.5). There is ample evidence to support that extract from *Cissus quadrangularis* exhibited strong antioxidant activity and free-radical scavenging effect in vitro systems. Furthermore, it may be recommended that the *Cissus quadrangularis* extracts possess antioxidant activities that makes them appropriate as a promising antidote which renders them suitable as potential therapeutics, thus making them excellent candidates for more detailed investigation. GC-MS analysis of methanolic extract of *Cissus quadrangularis* stem determined Thirty two compounds and the main constituents. Thus the results obtained confirm the therapeutic potency of *Cissus quadrangularis* used in traditional medicine. This forms a good basis for the selection of plant for further phytochemical and nutraceutical investigation.

## References

1. Aleryani SL, Cluette-Brown JE, Khan ZA, Hasaba H, Lopez de Heredia L, Laposata (2005) Fatty acid methyl esters are detectable in the plasma and their presence correlates with liver dysfunction. Clin Chim Acta 359:141-149.
2. Anonymous (2014b). www.dow.com/productsafety/assess/finder
3. Anonymous (2017). https://pubchem.ncbi.nlm.nih.gov/compound/linoleic_acid
4. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas MK (2012) Anti-Inflammatory Property of *n*-Hexadecanoic Acid: Structural Evidence and Kinetic Assessment. Chem Biol Drug Design 80(3):434-439.
5. Bao X, Katz S, Pollard M (2002) Ohlrogge Carbocyclic fatty acids in plants: Biochemical and molecular genetics characterization of cyclopropane fatty acid synthesis of Sterculiafoetida. Proc Natl Acad Sci USA 99: 7172-7177.

6. Bhujade AM, Talmale S, Kumar M, Gupta G, Reddanna P, Das SK (2012) Evaluation of Cissus quadrangularis extracts as an inhibitor of COX, 5-LOX, and proinflammatory mediators J Ethnopharmacol 141: 989-996.
7. Chidambara Murthy KN, Vanitha A, Mahadeva Swamy M, Ravishankar GA (2003) Antioxidant and Antimicrobial Activity of *Cissus quadrangularis* J Med Food 6(2):99-105.
8. Damge C, Hillarie D, Koenig M, Gross R, Hoeltzel A, Chapal J, Balboni G, Borg J, Ribes G (1995) Effect of n-Hexacosanol on insulin secretion in the rat. Eur J Pharmacol 274(1-3):133-139.
9. Devika GS (2019) Antioxidant activities and GC-MS analysis of ethanol extract of creeper stems of *Cissus quadrangularis* L. *J Pharmacogn Phytochem 8*(4): 760-765.
10. Dhanasekaran S (2020) Phytochemical characteristics of aerial part of *Cissus quadrangularis* (L) and its in-vitro inhibitory activity against leukemic cells and antioxidant properties Saudi J Biol Sci 27(5): 1302-1309.
11. Fernandes G, Banu J (2012) Medicinal properties of plants from the genus *Cissus*: A review. J Med Plants Res 6(16): 3080-3086.
12. Ghouse MS (2015) A Pharmacognostical Review on *Cissus Quadrangularis* Linn. Int Journal Pharm Biol 2 (7):28-35.
13. Hussain AZ, Kumaresan S (2013) GC-MS analysis and phytochemical screening of *Indoneesiella echioids (L.)Nees.* Arch Appl Sci Res 5 (5):159-162.
14. Manimekalai G, Kavitha S (2015) A study on properties of *Cissus quadrangularis* plant – A review. Int J Res Appl Soc Sci. 3 (6):15-18.
15. Mohanambal E, Shobana K, Sree SM, Kusuma GM, Satish K, Vijayakumar B (2011) Isolation of Alcoholic Extract of *Cissus quadrangularis* and Evaluation of *in-vitro* Anthelmintic Activity. Int J Novel Trends Pharm Sci. 1 (1):6-9.
16. Oyugi DA, Ayorinde FO, Gugssa A, Allen A, Izevbigie EB, Eribo B, Anderson WA (2011) Biological activity and mass spectrometric analysis of Vernonia amygdalina fractions. J Biosci Tech 2(3): 287-304.
17. Panthong A, Supraditaporn W, Kanjanpothi D, Reutrakul V (2007) Analgesic, anti-inflammatory and venotonic effects of *Cissus quadrangularis.* J Ethnopharmacol 110(2): 264-70.
18. Shinbori C, Saito M, Kinoshita Y, Satoh I, Kono T, Hanada T, Nanba E, Adachi K, Suzuki H, Yamada M. and Satoh K (2006) N-hexacosanol reverses diabetic induced muscarinic hypercontractility of ileum in the rat European J Pharmacol 545(2-3):177-184.