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**GC-MS PROFILE OF *CHLOROPHYTUM BORIVILIANUM* LEAVES AND  
ITS TOTAL PHENOLIC, TOTAL FLAVONOID CONTENT AND  
ANTIOXIDANT ACTIVITY**

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**ABSTRACT**

*Chlorophytum borivilianum* Santapau and Fernandes is a plant species reported from tropical wet forests in peninsular India, belongs to the Asparagaceae family, and is well known for its ethnomedicinal applications. In the present investigation, the methanol extract of *Chlorophytum borivilianum* leaves was analysed for total phenolic content, total flavonoid content and antioxidant activity moreover study also identify phytochemical constituents by GC-MS. The GC-MS analysis of the methanol extract of *Chlorophytum borivilianum* leaves detected the presence of 50 phytochemical constituents. Further, the results of DPPH show antioxidant activity at IC<sub>50</sub> value 369.6µg/mL, Total phenolic content 325.33±10.50 mg GAE/g and Total flavanoid content 534.6±57.0 mg QE/g mg QE/g. Results revealed that among the phytochemical constituents identified 9-Octadecenoic acid (Z)-, tetradecyl ester, 1-Monolinoleoylglycerol trimethylsilyl ether, Lycoxanthin, psi.,psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy reported for antioxidant

activity. The current study's findings will pave the road for the creation of herbal medications for a variety of disorders employing *Chlorophytum borivilianum* plants, perhaps leading to the production of therapeutics.

**Keywords:** *Chlorophytum borivilianum* , Antioxidant activity, GC-MS, Total phenolic content

## INTRODUCTION

Medicinal plants are used as a healthcare option by 80% of the world's population. People have relied on plants, dating back to 2800 BC. Herbal medications are the most rapidly expanding alternative treatments. The researcher needs to be explored medicinal plants and identify active ingredients and chemical constituents, using molecular biology and genomics, phytometabolites and metabolomics, that can sort out the battle for new medicines, and so on that can be imported as drugs, such studies might be broadened to include more in-depth assessments of traditional medicinal herbs [1]. *Chlorophytum borivilianum* Santapau and Fernandes (Liliaceae) belong to the Asparagaceae family, it is commonly known as Safed Musli in Hindi and an Indian spider plant in English. It is a 1.5-foot-long annual herb with thick, fascicled roots and spirally imbricate leaves. The flowers are densely grouped on the upper portion of the scape; the bracts are linear, papery, and purple, measuring 1-10, 5 cm in length; and the pedicle is white and 6–10 mm long. It produces green to yellow fruit with

roughly identical length and breadth. The seed is incredibly tiny and black, and it is surrounded by holes [2]. In Ayurveda *Chlorophytum borivilianum* is considered as 'Divya aushad' (divine medicine), it is defined with 'Caitha' as one of the sacred herbs. It belongs to the "Vajikaran Rasayana", a category which is a special group of Ayurvedic plants used for rejuvenating and revitalizing properties for improving sexual dynamics and alleviating sexual dysfunction [3]. *Chlorophytum borivilianum* possesses antitumor, antidiarrheal, analgesic, hypolipidemic, anti-inflammatory, antimicrobial, antistress, antioxidants, antidiabetic, and aphrodisiac activity [4]. The previous study reported phytochemicals such as saponins, alkaloids, flavonoids, and phenolic acids present in *C. borivilianum*. The study identified saponins, sapogenins, furostane and spirostane glycosides. The saponins, stigmasterol and echogenic are responsible for aphrodisiac, antioxidant, anticancer activities, and immune boosters. Furthermore, fructooligosaccharide and

inulin-type 2<sub>1</sub> linked fructans identified from *C. borivilianum* showed significant antidiabetic activity [5, 6]. As a result of the above-mentioned plant characteristics, it is worthwhile to screen the methanolic extract of *C. borivilianum* leaves for the presence of phytochemicals, identify and characterize phytoconstituents present in this plant using gas chromatography-mass spectrometric (GC-MS) analytic techniques, and evaluate its antioxidant potential using *in vitro* methods and determine its total phenolic and flavonoid content.

## MATERIALS AND METHODS

### Collection of plant material

*Chlorophytum borivilianum* leaves were collected in August from Jhansi (U.P). And authenticated by Dr J.C.Arya Research officer from Central Ayurveda Research Institute, CCRAS, Jhansi, (U.P). And we get the accession number 28756. The leaves were washed thoroughly with distilled water to remove dust and dried under the shade at room temperature until not get dried. The dried leaves were ground using the blender to obtain the coarse powder and kept in an airtight container till further use.

### Preparation of extracts

*C. borivilianum* dried leaves powder was extracted with 100% methanol by using the soxhlet apparatus at 50 °C at 24 h. The polar

extract was evaporated using a rotatory evaporator. And kept at 4°C.

### Phytochemical screening of Leaf extract

The freshly prepared crude methanolic extract of leaves was subjected to qualitative chemical tests to identify various classes of bioactive chemical constituents present in the leaves using standard procedures [7].

### Determination of total phenolic content

Folin-Ciocalteu reagent was used to determine the total phenolic content (TPC) of the various organic crude extracts. Gallic acid was used as a reference standard (65-1000 µg/mL) for plotting the calibration curve. A volume of 100µl of the plant extract/Gallic acid (1000 µg/mL) was mixed with 900 µL of 10% Folin-Ciocalteu reagent and was neutralized with 900 µl of sodium carbonate solution (7%, w/v). The reaction mixture was kept in dark at room temperature for 90 min with intermittent shaking for colour development. The absorbance of the resulting blue colour was measured at 765 nm. The TPCs were determined using a linear regression equation obtained from the standard plot of gallic acid. The content of total phenolic compounds was calculated as mean± SD ( $n=3$ ) and expressed as mg/g gallic acid equivalent (GAE) of dry extract [8].

### Estimation of total flavonoid content

In this method, quercetin was used as a standard to construct the calibration curve. Briefly, 1 mg of quercetin was dissolved in methanol and then diluted to 35,65,125,250, 500, and 1000 µg/mL. The diluted standard solutions of plant extracts (0.5 mL) of different concentrations were separately mixed with 3.4 ml of 30% methanol, 0.15 mL of 10% aluminium chloride, 0.15 mL of 0.5M of NaNO<sub>2</sub> mol/L after 5 min incubation add 1ml of NaOH (1M). The absorbance of the reaction mixture was measured at 506 nm. The amount of flavonoid was calculated from the linear regression equation obtained from the quercetin calibration curve. The flavonoid content was calculated as mean±SD ( $n=3$ ) and expressed as mg/g of quercetin equivalent (QE) of dry extract [9].

#### Assay of free radical scavenging activity by the DPPH method

The free radical scavenging activity of different concentrations of crude extracts of *C. borivilianum* leaves and standard ascorbic acid was evaluated by using DPPH radical scavenging method as per the reported method [10]. The percentage of radical scavenging activity of tested extracts and positive control ascorbic acid was calculated by using the following formula:

$$\text{Free radical scavenging activity (\%)} = 100 - \left[ \frac{(A_s - A_b)}{A_c} \times 100 \right]$$

Where  $A_b$  = Absorbance of blank at 517 nm and  $A_s$  = Absorbance of the sample.

The concentration of sample required to scavenge 50% of DPPH free radical (IC<sub>50</sub>) was determined using a graph prism.

#### GC-MS analysis

GC-MS analysis of a crude extract of *C. borivilianum* leaves was performed on a PerkinElmer Clarus 600 GC System, fitted with a capillary column (30.0m x 250µm), transfer temperature, 200 °C, Ultra-high purity helium (99.99%) was used as carrier gas at a constant flow rate of 1.0 mL/min. The injection, temperatures were all 250 °C. The oven temperature was programmed from 40 °C (hold for 5 min), ramp 12°C/min to 260°C, and hold for 10min. The crude samples were diluted with the appropriate solvent (1/100, v/v) and filtered. The particle-free diluted crude extracts (1 µL) were taken in a syringe and injected into the injector with a split ratio of 50:1. All data were obtained by collecting the full-scan mass spectra within the scan range of 50-500 Da. The percentage composition of the crude extract constituents was expressed as a percentage by peak area.

## RESULTS AND DISCUSSION

### Preliminary phytochemical screening

The preliminary screening confirmed the presence of various classes of bioactive chemical constituents in the methanolic

extract of *C.borivilianum* leaves. Numerous reports are available on saponin fraction in *C.borivilianum* and its antimicrobial activity, aphrodisiac, immune-modulatory, and anticancer activities. However, less literature is available on the antioxidant potential of

*C.borivilianum* leaves. The phytochemical analysis showed the presence of major classes of secondary metabolites such as tannins, alkaloids, flavonoids, cardiac glycosides etc. (Table 1) whereas proteins and amino acids were found to be absent in the extract.

**Phytochemical Profiling Of *Chlorophytum borivilianum***

Phytochemical Test	
Tests	<i>Chlorophytum borivilianum</i> Leaf
<b>Alkaloid Test</b>	
Mayner's Test	+
Wagner's Test	+
Phenol	+
Tannin	+
Saponin	+
<b>Glycoside</b>	
Liebermann's Test	+
Salkowski's Test	+
Steroid	+
Terpenoids	+
Flavanoids	+
<b>Carbohydrate</b>	
Molish's Test	-
<b>Amino acids</b>	
Millon's Test	-
Biuret Test	-
Proteins	-

### TPCs

The TPC of the various leaf extract is expressed in terms of GAE. The TPCs were calculated using the following linear regression equation obtained from the standard plot of gallic acid:  
 $y=0.0004x+0.0549, r^2=0.983$

Where y is absorbance and x is the amount of Gallic acid in  $\mu\text{g}$ .

The TPC obtained from the methanolic extract of *C. borivilianum* is  $325.33\pm 10.50$  mg GAE/g respectively.

### TFCs

The TFCs of the various crude extracts are expressed in terms of QE. The TFCs were calculated using the following linear regression equation obtained from the standard plot of quercetin:

$$y=0.0011x+0.2242, r^2=0.894$$

Where y is absorbance and x are the amount of quercetin in  $\mu\text{g}$ .

The TFC obtained from the methanolic extract of *C. borivilianum* is  $534.6\pm 57.0$  mg QE/g respectively.

### In vitro antioxidant activity

The antioxidant activity of leaf extracts on the DPPH was expressed as % inhibition and was compared with the standard antioxidant, ascorbic acid. The extracts showed a dose-dependent scavenging activity of DPPH comparable to standard antioxidants. The  $IC_{50}$  value of the extract obtained is  $369.6\mu\text{g/mL}$  as compared to the ascorbic acid  $IC_{50}$  value of  $80.20\mu\text{g/ml}$  (Figure 1).

### GC-MS Analysis

The GC-MS spectra analysis of *C. borivilianum* methanol extract of leaves

reveals 7 peaks, depicted in Figure 2. The presence of 50 distinct bioactive chemicals, specifically 9-Octadecenoic acid (Z)-, tetradecyl ester, 1-Monolinoleoylglycerol trimethylsilyl ether, Lycoxanthin, psi.,psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy compounds were identified that exhibit antioxidant activity, were identified as significant compounds in this study. The stated therapeutic activity of these discovered bio-compounds is shown in Table 2.

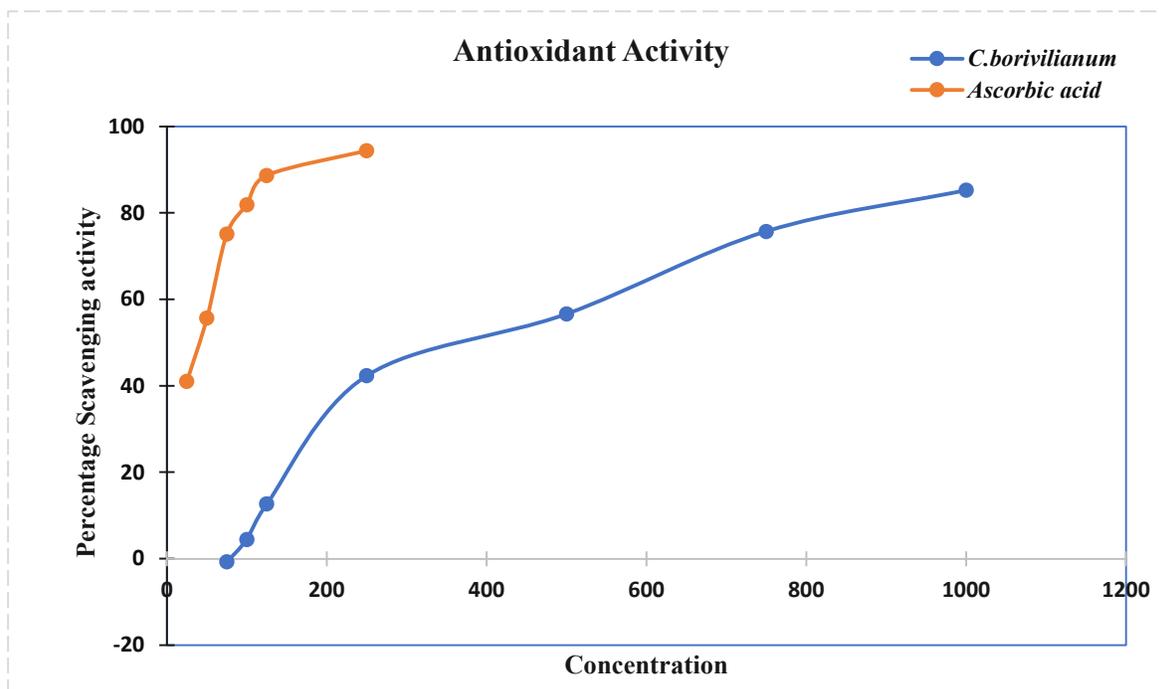


Figure 1: Percentage scavenging activity graph

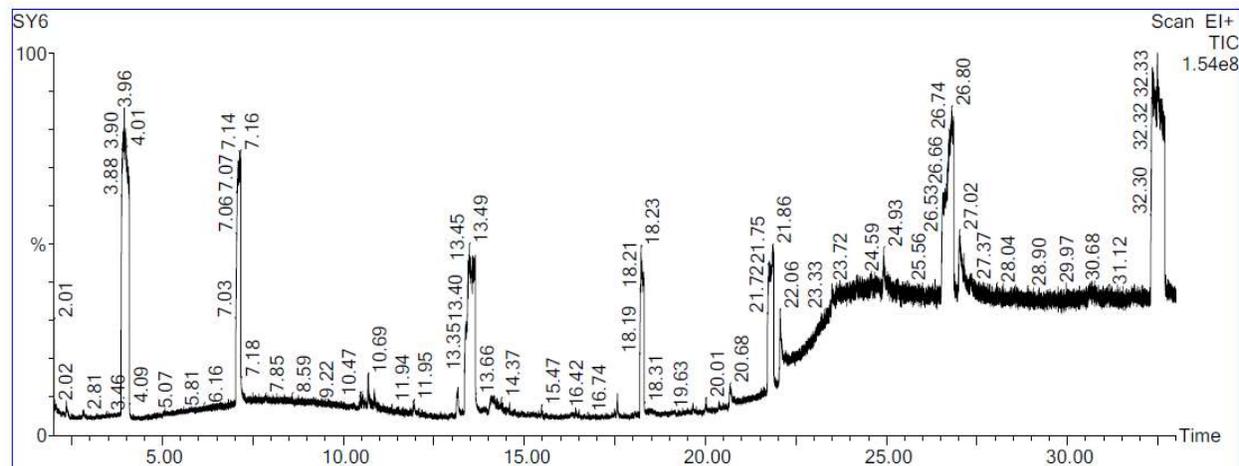
Figure 2: Gas chromatogram of *C. borivilianum* methanolic leaf extract

Table 2: Chemical composition of extracts by GC-MS analysis

Sr.No	RT	PP%	Compound Name	M.F	M.W
1	26.800	6.045	psi.,psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy	C <sub>42</sub> H <sub>64</sub> O <sub>2</sub>	601 g/mol
			Cholestane, 3,5-dichloro-6-nitro-, (3 $\alpha$ ,5 $\alpha$ ,6 $\alpha$ )-	C <sub>27</sub> H <sub>45</sub> C <sub>12</sub> NO <sub>2</sub>	486.6 g/mol
			.psi.,psi.-Carotene, 3,4-didehydro-1,2,7',8'-tetrahydro-1-methoxy-2-oxo	C <sub>41</sub> H <sub>58</sub> O <sub>2</sub>	582.9 g/mol
			9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingo (5,6,7-Triacetoxy-4b,8-dimethyl-2-oxo-tetradecahydro-phenanthren-1-yl)-acetic acid, methyl ester	C <sub>28</sub> H <sub>40</sub> O <sub>10</sub> C <sub>25</sub> H <sub>36</sub> O <sub>9</sub>	536.6 g/mol 480.5 g/mol
2	32.488	2.187	Prosta-5,13-dien-1-oic acid, 9,11,15-tris[(trimethylsilyloxy)-, trimethylsilyl ester, (5Z,9 $\alpha$ ,11 $\alpha$ ,13E,15S)-	C <sub>32</sub> H <sub>66</sub> O <sub>5</sub> Si <sub>4</sub>	643.2 g/mol
			(25S)-3Beta-acetoxy-5alpha,22beta-spirost-9(11)-en-12beta-ol	C <sub>29</sub> H <sub>44</sub> O <sub>5</sub>	472.7 g/mol
			Cholest-2-ene-2-carbothioic acid, 3-hydroxy-, O-ethyl ester, (5 $\alpha$ )-	C <sub>30</sub> H <sub>50</sub> O <sub>2</sub> S	474.8 g/mol
3	32.349	1.634	1,3-Dichloro-1,3-bis(norbornadien-2-yl)-1,3-bis(3trimethylsilylpropyl)disiloxane	Not reported	Not reported
			L-Valine, N-[N,O-bis(2,4-dinitrophenyl)-L-tyrosyl]-, methyl ester	C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>	117.15 g/mol
4	27.016	1.452	Octadecanal, 2-bromo-	C <sub>18</sub> H <sub>35</sub> BrO	347.4 g/mol
			Cyclohexane, 1,1'-dodecylidenebis[4-methyl-Monoelaidin	C <sub>26</sub> H <sub>50</sub> C <sub>21</sub> H <sub>40</sub> O <sub>4</sub>	362.7 g/mol 356.5 g/mol
			cis-13-Eicosenoic acid	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	310.5 g/mol
			11,13-Dimethyl-12-tetradecen-1-ol acetate	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.5 g/mol
5	32.609	1.421	2 $\alpha$ ,4 $\alpha$ -Epoxyethylphenanthrene-7-methanol, 1,1-dimethyl-2-methoxy-8-(1,3-dithiin-2-ylidene)methyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-, acetate	C <sub>27</sub> H <sub>38</sub> O <sub>4</sub> S <sub>2</sub>	490.7 g/mol
			3'H-Cycloprop(1,2)cholesta-1,4,6-trien-3-one, 1'-carboethoxy-1'-cyano-1 $\alpha$ ,2 $\alpha$ -dihydro	C <sub>32</sub> H <sub>49</sub> NO <sub>3</sub>	495.7 g/mol
			9-Desoxy-9x-chloroingol 3,7,8,12-tetraacetate	C <sub>28</sub> H <sub>39</sub> ClO <sub>9</sub>	555.1 g/mol
			1',1'-Dicarboethoxy-1 $\alpha$ ,2 $\alpha$ -dihydro-3'H-cycloprop[1,2]cholesta-1,4,6-trien-3-one	Not reported	Not reported

6	32.648	1.053	<b>Hematoporphyrin</b>	<b>C<sub>34</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub></b>	<b>598.7g/mol</b>
			8-[2-(2-Acetylamino-phenyl)-2-oxoethyl]-3-isopropyl-6a,7,10b-trimethyl-dodecahydrobenzo[f]chromene-7-carboxylic acid, methyl ester	Not reported	Not reported
			3'H-Cycloprop(1,2)-5-cholest-1-en-3-one, 1'-carboethoxy-1'-cyano-1,2-dihydro	C <sub>32</sub> H <sub>49</sub> NO <sub>3</sub>	495.7 g/mol
			17-(1,5-Dimethylhexyl)-10,13-dimethyl-3-styrylhexadecahydrocyclopenta[a]phenanthren-2-one	Not reported	Not reported
7	7.161	3.766	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-2-chloro-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-, [1aR-(1aà,1bá,2à,3á,4aá,7aà,7bà,8à,9á,9aà)]-	C <sub>28</sub> H <sub>37</sub> ClO <sub>11</sub>	548.6g/mol
			<b>Lycoxanthin</b>	<b>C<sub>40</sub>H<sub>56</sub>O</b>	<b>552.9 g/mol</b>
8	21.765	1.328	Tungsten, pentacarbonyl(4,5-diethyl-2,2,3-trimethyl-1-phenyl-1-phospho-2-sila-5-boracyclohex-3-ene-P1)-, (oc-6-22)-	Not reported	Not reported
			<b>Lycophyll</b>	<b>C<sub>40</sub>H<sub>56</sub>O<sub>2</sub></b>	<b>568.9 g/mol</b>
			Oleic acid, eicosyl ester	C <sub>38</sub> H <sub>74</sub> O <sub>2</sub>	563 g/mol
			1-Monolinoleoylglycerol trimethylsilyl ether	Not reported	Not reported
			Dasycarpidan-1-methanol, acetate (ester)	C <sub>20</sub> H <sub>26</sub> N <sub>2</sub> O <sub>2</sub>	326.4 g/mol
			9-Octadecenoic acid (Z)-, tetradecyl ester	C <sub>32</sub> H <sub>62</sub> O <sub>2</sub>	478.8 g/mol
			2-(16-Acetoxy-11-hydroxy-4,8,10,14-tetramethyl-3-oxohexadecahydrocyclopenta[a]phenanthren-17-ylidene)-6-methyl-hept-5-enoic acid, methyl ester	C <sub>32</sub> H <sub>48</sub> O <sub>6</sub>	528.7 g/mol
			4-Piperidineacetic acid, 1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1H-indol-2-yl]-à-methyl-, methyl ester	C <sub>7</sub> H <sub>13</sub> NO <sub>2</sub>	143.18 g/mol
			<b>Tribehenin</b>	<b>C<sub>69</sub>H<sub>134</sub>O<sub>6</sub></b>	<b>1059.8 g/mol</b>
			Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate (ester)	C <sub>22</sub> H <sub>28</sub> N <sub>2</sub> O <sub>3</sub>	368.5 g/mol
			17-Pentatriacontene	C <sub>35</sub> H <sub>70</sub>	490.9 g/mol
9	26.559	0.983	1,3-Dichloro-1,3-bis(norbornadien-2-yl)-1,3-bis(3-trimethylsilylpropyl)disiloxane	Not reported	Not reported
			<b>Rhodoxanthin</b>	<b>C<sub>40</sub>H<sub>50</sub>O<sub>2</sub></b>	<b>562.8 g/mol</b>
			1,8,15,22-Tetraaza-2,7,16,21-cyclooctacosanetetrone	C <sub>24</sub> H <sub>44</sub> N <sub>4</sub> O	452g/mol
			Cholest-2-eno[2,3-b]quinoxaline, 6'-nitro-	C <sub>33</sub> H <sub>47</sub> N <sub>3</sub> O <sub>2</sub>	517.7g/mol
			7,8-Epoxyanostan-11-ol, 3-acetoxy-	C <sub>32</sub> H <sub>54</sub> O <sub>4</sub>	502.8 g/mol
			Aconitane-1,7,8,14-tetrol, 20-ethyl-6,16-dimethoxy-4-(methoxymethyl)-, 14-acetate, (1à,6á,14à,16á)-	C <sub>24</sub> H <sub>39</sub> NO <sub>7</sub>	495.6g/mol
			Pregn-4-en-18-al, 3-(methoxyimino)-20-oxo-11,21-bis(trimethylsilyloxy)-, 18-(O-methylxime), (11á,17à)-	C <sub>29</sub> H <sub>50</sub> N <sub>2</sub> O <sub>5</sub> Si <sub>2</sub>	562.9 g/mol
10	32.455	0.591	Molybdenum, tricarbonyl-[N-butyl-bis(2-(butylphosphino)ethyl)amine]	MO	96 g/mol
			4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one, 8,8abis(acetyloxy)-2a-[(acetyloxy)methyl]-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8adodecahydro-	C <sub>26</sub> H <sub>34</sub> O <sub>11</sub>	522.5 g/mol

11	24.750	0.576	3,3a,6b-trihydroxy-1,1,5,7-tetramethyl-		
			Haloxazolam	$C_{17}H_{14}BrFN_2O_2$	377.2 g/mol
			1,2-Cinnolinedicarboxylic acid, 1,2,3,5,6,7,8,8a-octahydro-4-trimethylsilyloxy-, diethyl ester	$C_{17}H_{30}N_2O_5Si$	370.5 g/mol
			Endrin	$C_{12}H_8Cl_6O$	380.9g/mol
			3-Desoxo-3,16-dihydroxy-12-desoxyphorbol 3,13,16,20-tetraacetate	$C_{28}H_{38}O_{10}$	534.6 g/mol
			9,19-Cyclolanostane-6,7-dione, 3-acetoxy	$C_{32}H_{50}O_4$	498.7 g/mol
Acetic acid, 17-(4-chloro-5-methoxy-1,5-dimethylhexyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1-phenanthryl-	$C_{33}H_{55}ClO_3$	535.2 g/mol			

Plants have a plethora of therapeutics and phytoconstituents. Some of these secondary metabolites from plants are rich in natural antioxidants. Free radicals have been linked to a wide range of clinical symptoms. Antioxidants attack free radicals and protect us from a variety of ailments such as atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia and degenerative eye disease. They work by either scavenging reactive oxygen species or strengthening antioxidant defence systems. Plant phenolic compounds are an important source of hydroxyl groups that provide scavenging capacity. Phenolic compounds are classified into various groups, the most important of which are flavonoids, which have strong antioxidant properties. Flavonoids are very efficient scavengers of most oxidising chemicals, including singlet oxygen and other free radicals linked to a variety of illnesses [11].

However, less literature is available on the antioxidant potential of *C. borivilianum* leaves. The total phenolic content (TPC) in roots of *C. borivilianum* reported was 4.9 mg/g GAE (Gallic acid equivalent) and showed IC<sub>50</sub> (Inhibitory Concentration) 4.96 mg/ml of free radical scavenging activity [12]. However, the leaf in this study shows antioxidant activity at an IC<sub>50</sub> value of 369.6µg/mL and the Total phenolic content of 325.33±10.50 mg GAE/g. Therefore, based on the phytochemical screening results, the total phenolic and flavonoid contents of the methanolic extract of *C. borivilianum* leaves were estimated and also its antioxidant potential was investigated by *in vitro* DPPH methods. Although to identify the chemical compounds GC-MS analysis was performed. A total of 7 peaks was observed, and 9-Octadecenoic acid (Z)-, tetradecyl ester, 1-Monolinoleoylglycerol trimethylsilyl ether, Lycoxanthin, psi.,psi.-

Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy compounds have identified that exhibit antioxidant activity. Thus, it can be concluded that the antioxidant activity of this plant is due to the presence of phytoconstituents.

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