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DPPH Radical Scavenging Activity of Corydine Isolated from *Tinospora cordifolia*

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Abstract

Reactive Oxygen Species (ROS) are involved in the pathogenesis of several diseases, and antioxidants are important for maintaining optimal human health and well-being because of their role in scavenging free radicals. The aim of this study is to evaluate the antioxidant potentials of compound isolated from *Tinospora cordifolia*. Crude extract of *T. cordifolia* stem was successively partitioned into n-hexane, dichloromethane, ethyl acetate and aqueous fractions. Standard procedures were used to evaluate the (1,1-diphenyl-2-picrylhydrazyl) DPPH radical scavenging activity *in vitro*. Most active fraction was subjected to chromatographic method to isolate and purify compound. The compound was characterized using spectroscopic analyses (EI-MS, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR, FT-IR, UV). Ethyl acetate fraction had the highest radical scavenging activity with IC_{50} of 0.419±0.03 mg/mL among tested extracts as compared to standard drug vitamin C (IC_{50} = 0.008±0.00 mg/mL). The compound Corydine, an isoquinoline alkaloid was identified and was able to scavenge the DPPH radical with IC_{50} of 0.298±4.38 mM (67.2% inhibition) as compared to N-Acetyl-L-Cysteine (IC_{50} = 0.115±3.91 mM, 97.5% inhibition) at 0.5 mM concentration. Corydine can possibly mitigate cellular damage caused by reactive oxygen species and help to maintain optimum health and wellbeing.

Keywords: *Isoquinoline alkaloid, Corydine, Antioxidant, Tinospora cordifolia, Chromatography*

1. Introduction

Oxidative stress caused by free radicals is a worldwide problem today due to its debilitating effects on human health. Free radicals are involved in the development of several diseases such as Inflammatory diseases

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(Sreejayan and Rao, 1996), cardiovascular diseases, cancer (Gerber *et al*., 2002), neurodegenerative diseases such as Alzheimer's disease (Di Matteo and Esposito, 2003).

Antioxidant compounds scavenge free radicals from the body's cells to prevent or reduce oxidative damage. Most of these antioxidants are found in natural plant sources that can quench reactive free radicals (Knekt *et al*., 1996). Plants and their active ingredients have been the basis of traditional medicines around the world for thousands of years, and continue to provide new remedies for mankind (Winston, 1999). The polyphenol-rich active fraction of *Acacia arabica* is a potent radical scavenger and has been reported to protect against lipid peroxidation by tert-butyl hydroperoxide (TBH) (Sundaram and Mitra, 2007). Both aqueous and ethanolic extracts of ginger (*Zingiber officinale*) have been reported to have significant natural antioxidant activity. Therefore, ginger consumption may help reduce the progression of various oxidative stress diseases (Morakiyo *et al*., 2011). Evaluation of antioxidant activity *in vitro* indicates that methanol, ethanol, acetone, and water extracts of *Pseuderanthemum palatiferum* exhibit DPPH radical scavenging activity, reducing activity, inhibitory activity on lipid peroxidation, and protection-induced hemolysis (Chayarop *et al*., 2011). In a study by Kery *et al*. (2003) demonstrated that *Sempervivum tectorum* extract possesses true superoxide scavenging activity in a cell-free system by DPPH assay. This indicates its ability to reduce oxidative damage and enhance defense mechanisms against free radicals. Cabera *et al*. (2006) reported that (-)-epigallocatechin-3-gallate (EGCG) isolated from *Camellia sinensis* leaves is a potent antioxidant that can protect erythrocyte membrane bound ATPases against oxidative stress and enhanced blood antioxidant potential leads to reduced oxidative damage in macromolecules such as DNA and lipids. Curcumin is a potent antioxidant isolated from the rhizome of *Curcuma longa*. It may scavenge the epoxides and prevent binding to macromolecules (Braga *et al*., 2003). Zingiberene the main terpenoid from ginger (*Zingiber officinale)* extract may help combat the progression of various diseases with an oxidative stress component such as atherosclerosis, diabetes mellitus (Morakinyo *et al*., 2011).

Tinospora cordifolia (Menispermaceae) is widely used in indigenous systems of medicine. It has anti-cancer, anti-diabetes (Pandey *et al*., 2012), neuroprotective (Kosaraju and Roy, 2014), anti-ulcer (Kaur *et al*., 2014), immune stimulating (Gupta *et al*., 2011) and anagelsic activity (Goel and Pathak, 2014).

The purpose of this study was to evaluate the antioxidant capacity of extracts and compound isolated from *Tinospora cordifolia*.

2. Materials and Methods

2.1. Collection, Authentication and Extraction of Plant Material

Tinospora cordifolia stem was collected, identified by a taxonomist and authenticated at Forest Research Institute of Nigeria where voucher specimen was deposited with number FHI 112287. The dried powdered stem was macerated with methanol for 72 h. The extract was then filtered, concentrated *in vacuo* at 40 °C optimum temperature under reduced pressure using a rota evaporator. The methanol crude extract was further partitioned into n-hexane, dichloromethane, ethyl acetate and aqueous fraction using a separating funnel.

2.2. Source of Compound

Corydine, an isoquinoline alkaloid (Figure 1) was isolated as crystals and characterized using spectroscopic analyses such as ^{1}H-NMR, ^{13}C-NMR, EI-MS, FT-IR, UV (see Appendix 1) (Onoja *et al*., 2020).

2.3. DPPH Radical Scavenging Activity

The scavenging capacity of the extracts and compound uses the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) as described by Brand-Williams *et al.* (1995). To 1ml of various concentrations (5 - 0.15625 mg/mL of the plant extracts/0.5 mM of compound) or standard (vitamin C/N-Acetyl-L-Cysteine) in test tubes was added 1 mL of 0.3 mM DPPH in methanol. The mixture was mixed and incubated in the dark for 30 min before reading the absorbance at 517 nm against a DPPH control containing only 1 mL of methanol instead of the test sample.

The percent of inhibition was calculated in following way:

$$I\% = [(A_{blank}-A_{sample})/A_{blank}] \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test samples), and A_{sample} is the absorbance of the test samples. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration.

2.4. Statistical Analysis

All data will be expressed as mean ± S.D. and of triplicate parallel measurements. Standard curves were generated and calculation of the 50% inhibitory concentration (IC_{50}) values was done using Microsoft Excel.

3. Results and Discussion

The plant kingdom provides a wide range of secondary metabolites, especially plant polyphenols, with antioxidant potential that may protect against various diseases associated with oxidative stress and free radical damage (Teixeira *et al.*, 2013). The DPPH test (1,1-diphenyl-2-picrylhydrazyl radical) is a widely used

Table 1: DPPH Radical Scavenging Activity (RSA) Activity of Crude Extract and Fractions of *Tinospora cordifolia* at 5-0.15625 mg/mL

Test Sample(s)	Crude and Fraction(s)	DPPH RSA IC_{50}±SD (mg/mL)
Tinospora cordifolia stem	n-hexane	0.458±0.02
	Dichloromethane	1.210±0.01
	Ethyl acetate	0.419±0.03
	Aqueous	0.587±0.02
	Crude	1.099±0.19
	Vitamin C	0.008±0.00

Note: Values are presented as mean ± standard deviation (n = 3).

Table 2: DPPH Radical Scavenging Activity of Corydine

Compound(s)	Conc. (mM)	% Inhibition	IC_{50}±SEM (mM)
Corydine	0.5	67.2	0.298±4.38
N-Acetyl-L-Cysteine (standard)	0.5	97.5	0.115±3.91

Note: Values are presented as mean ± standard error of mean (n = 3).

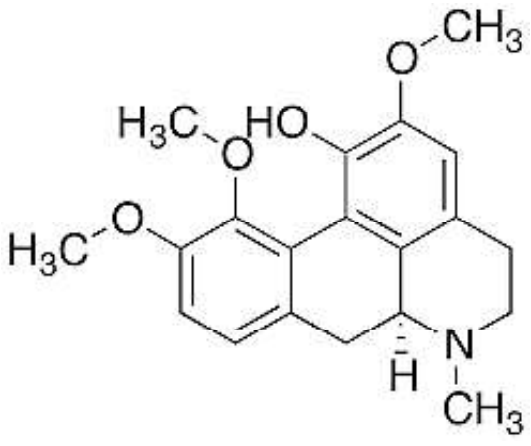

Figure 1: Corydine

method to assess the radical scavenging activity of plant extracts and compounds. This method is based on the reduction of a methanolic DPPH solution in the presence of an antioxidant, the reaction forming non-radical DPPH-H. Stable DPPH was reduced by extracts/compounds, changing color from purple to yellow to varying degrees depending on the presence of antioxidant compounds. The degree of discoloration designates the scavenging power of the extracts and compound isolated. DPPH radical scavenging based antioxidant potential of the extracts was assessed using the IC_{50} parameter. Here, IC_{50} means the concentration of antioxidant required to scavenge 50% of the 1,1-diphenyl-2-picrylhydrazyl radicals in the specified time. The lower the IC_{50} value, the higher antioxidant activity of the plant extracts/compound (Prieto *et al.*, 1999). In DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging test at 5-0.15625 mg/mL, ethyl acetate fraction of *Tinospora cordifolia* stem has the highest scavenging capacity (IC_{50} of 0.419±0.03 mg/mL) among tested extracts as compared to the standard vitamin C with IC_{50} of 0.008±0.00 mg/mL (Table 1). The compound Corydine was able to scavenge the DPPH radical with IC_{50} of 0.298±4.38 mM (67.2% inhibition) as compared to N-Acetyl-L-Cysteine (IC_{50} =0.115±3.91 mM) with 97.5% inhibition at 0.5 mM concentration, respectively (Table 2). Ethyl acetate fraction and the isoquinoline alkaloid, corydine was able to scavenge the 1,1-diphenyl-2-picrylhydrazyl radical which could be due to its ability to donate hydrogen atom thereby reducing the radical. It can also be concluded that phenolic hydroxyl group (-OH) and methoxyl group ($-OCH_3$) in the compound could be responsible for its anti-radical activity (Farhoosh *et al.*, 2016; Chen *et al.*, 2020). To corroborate my findings, two bisbenzylisoquinoline alkaloids from *Stephania rotunda*: cepharanthine and fangchinoline demonstrated antioxidant activity (Ilhami *et al.*, 2010). Denudatine-type C_{20}-diterpenoid alkaloids with vicinal-triol system (handelidine, cochlearenine, yesoxine) and benzyltetrahydroisoquinoline alkaloids ((+)-*N*-methylcoclaurine and (+)-orientaline) isolated from the roots of *Aconitum handelianum* exhibited significant antioxidant activities (Tian-Peng *et al.*, 2016).

4. Conclusion

Tinospora cordifolia extracts and the antioxidant compound (corydine) can mitigate cellular damage caused by reactive oxygen species which is critical for maintaining optimal health and wellbeing.

Conflicts of Interest

Authors declare no conflict of interest

Acknowledgment

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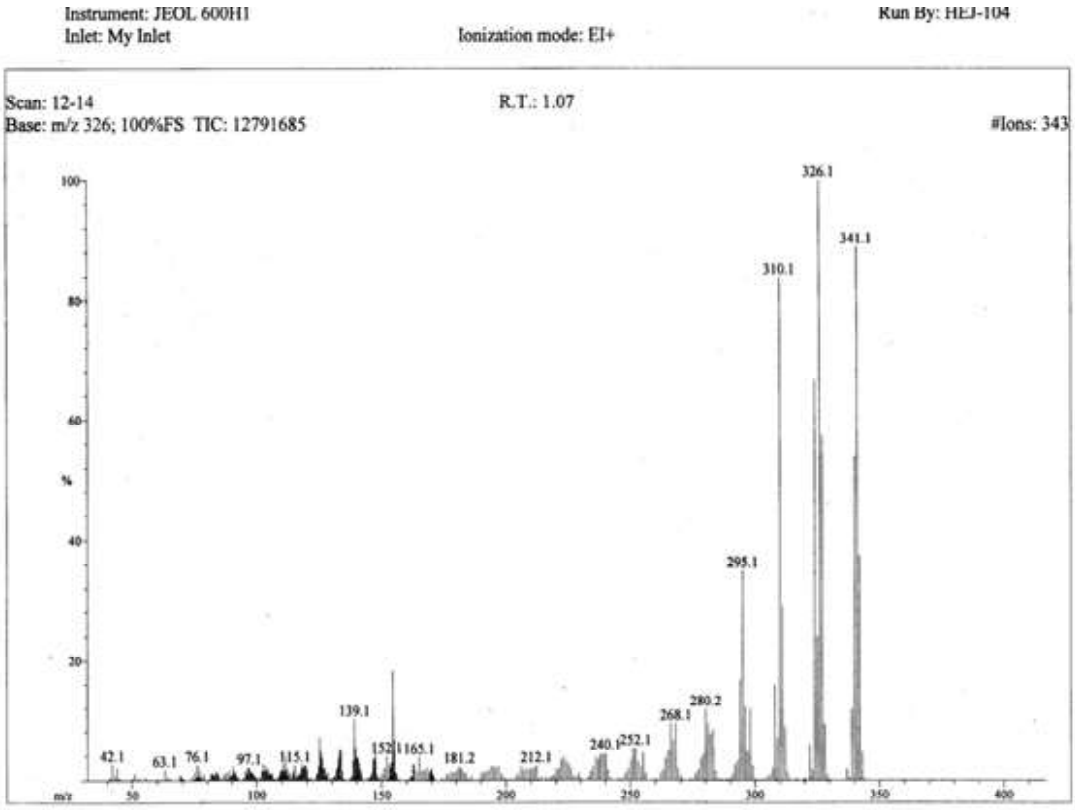
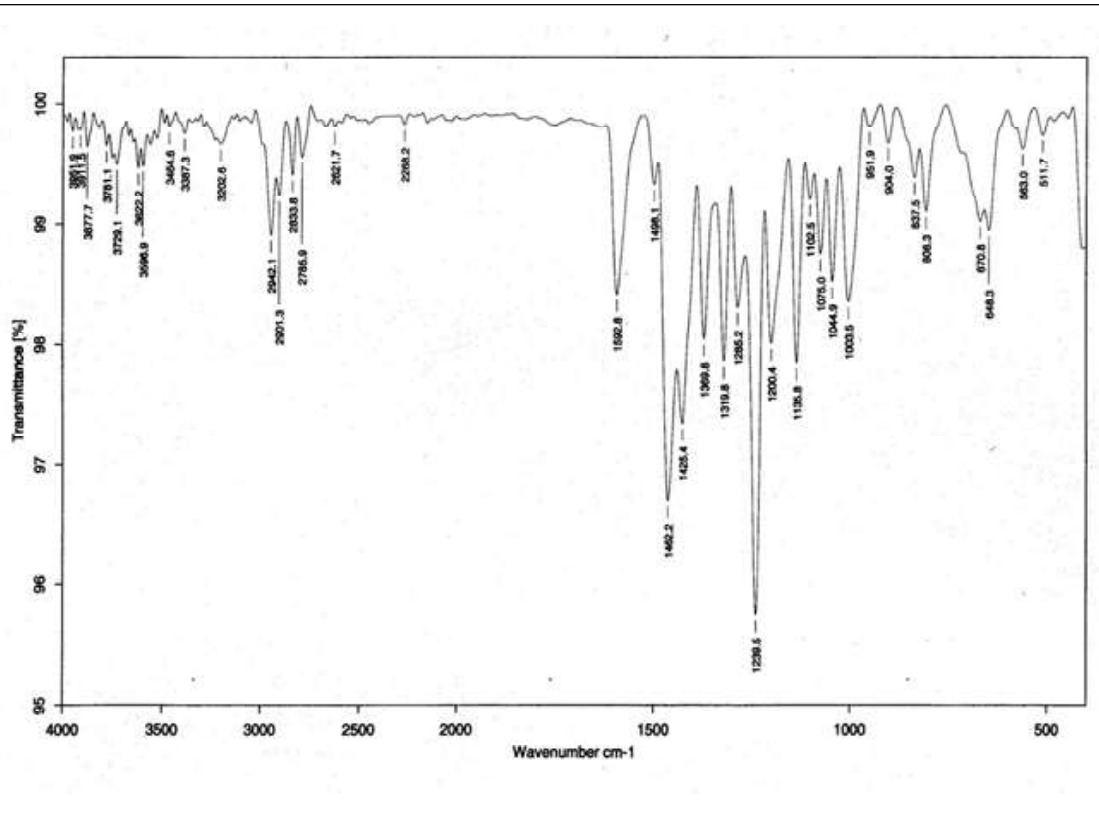
Appendix 1: Spectroscopic Analyses of Corydine



EI-MS Spectra of Corydine



FT-IR Spectra of Corydine (Liquid Technique)

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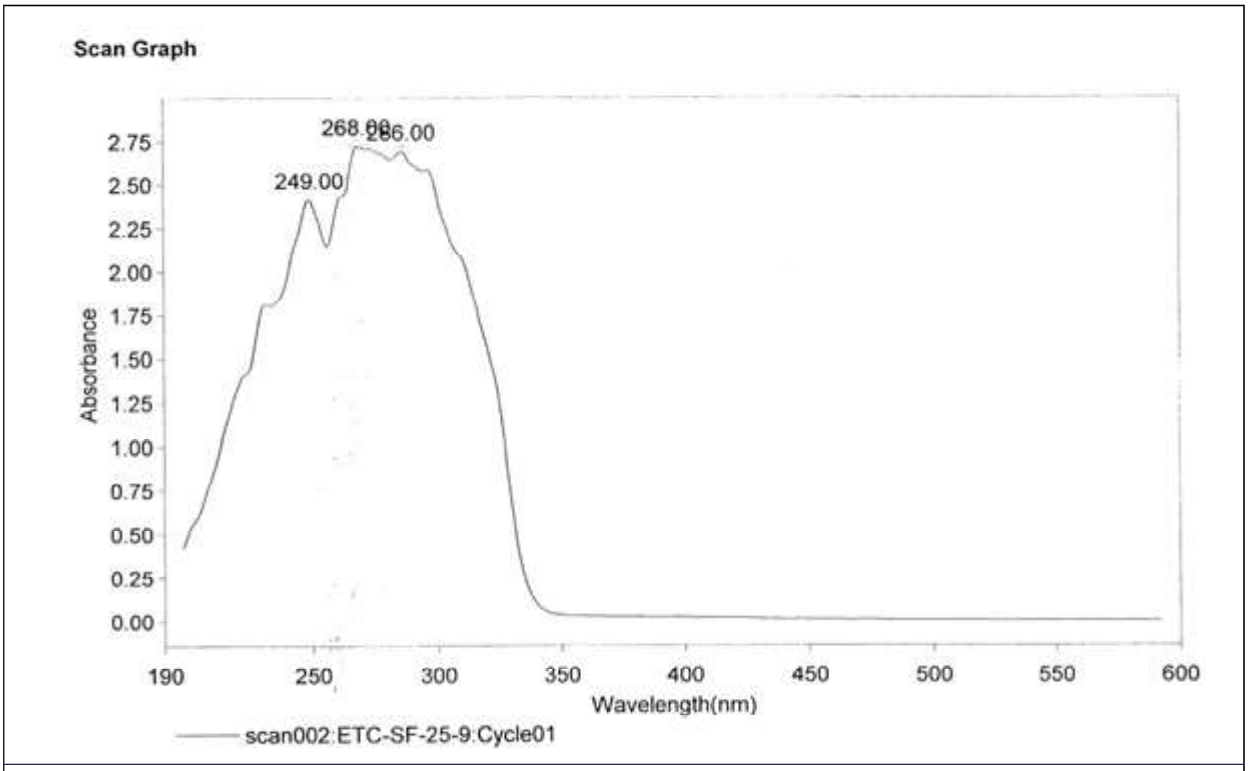
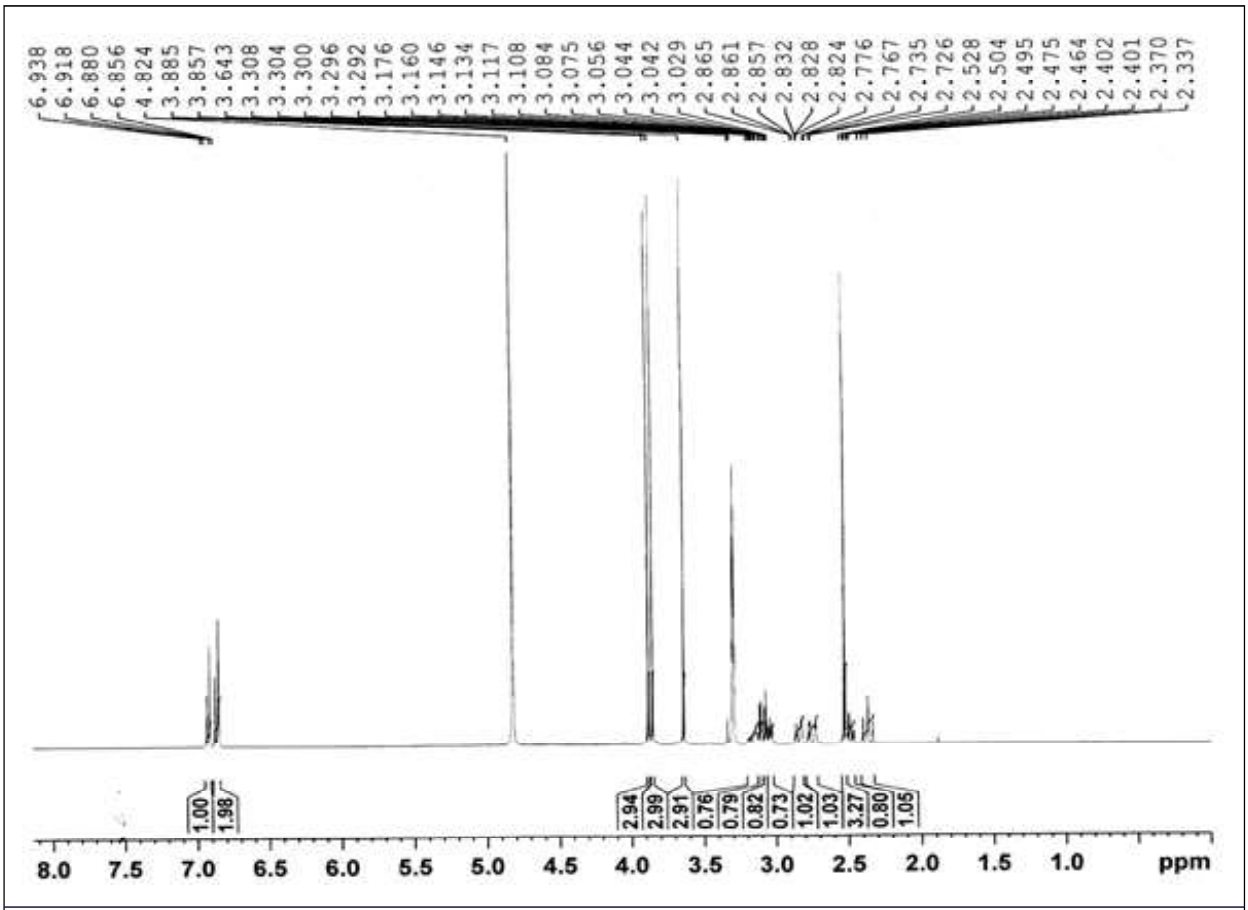


UV Spectra of Corydine



H-NMR Spectra of Corydine

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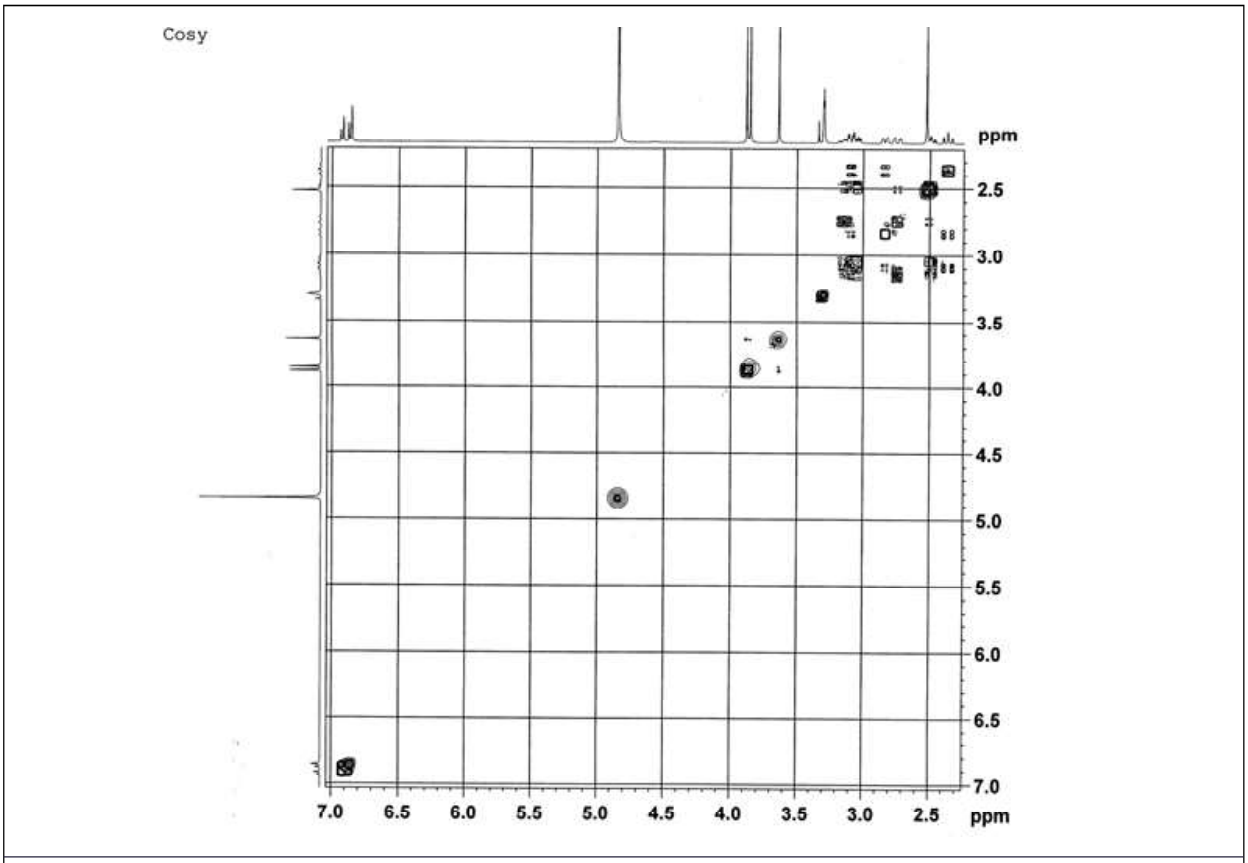
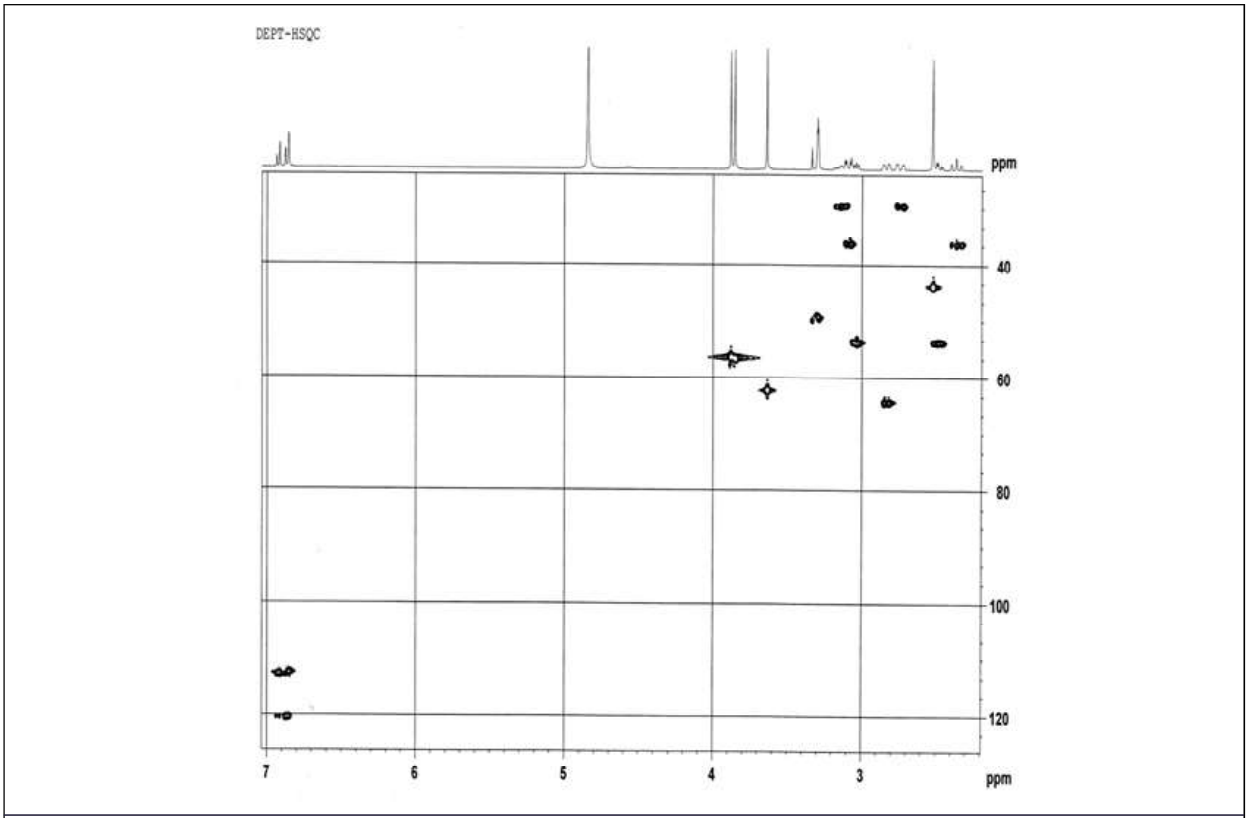
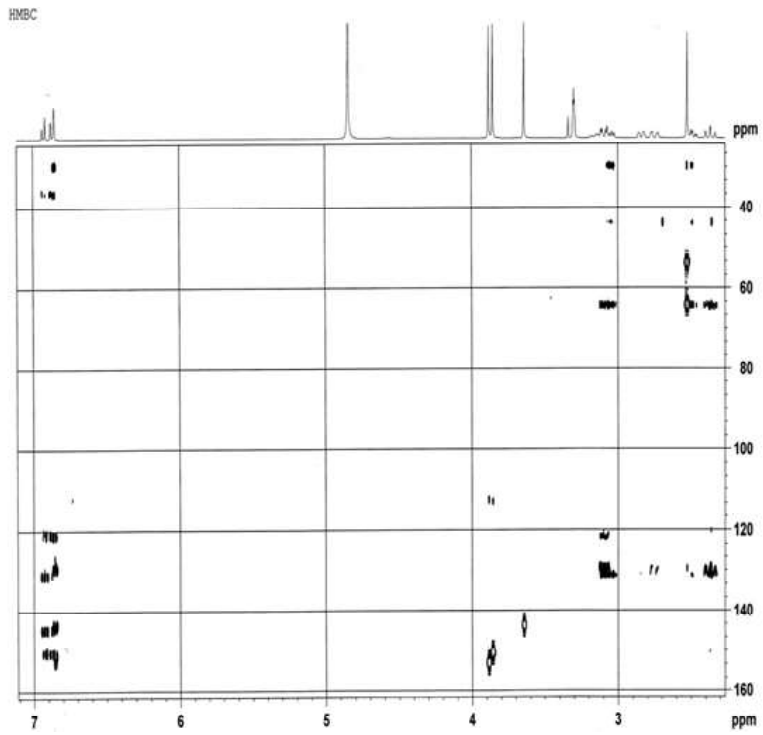


COSY Spectra of Corydine

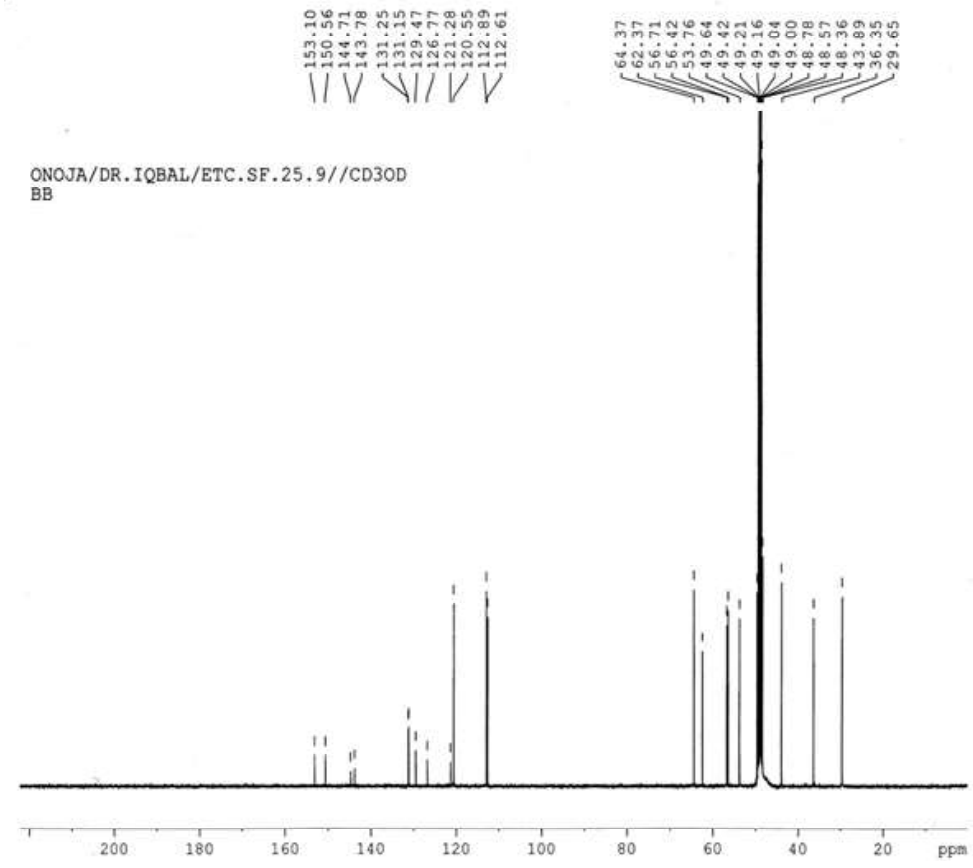


DEPT-HSQC Spectra of Corydine

Appendix 1 (Cont.)

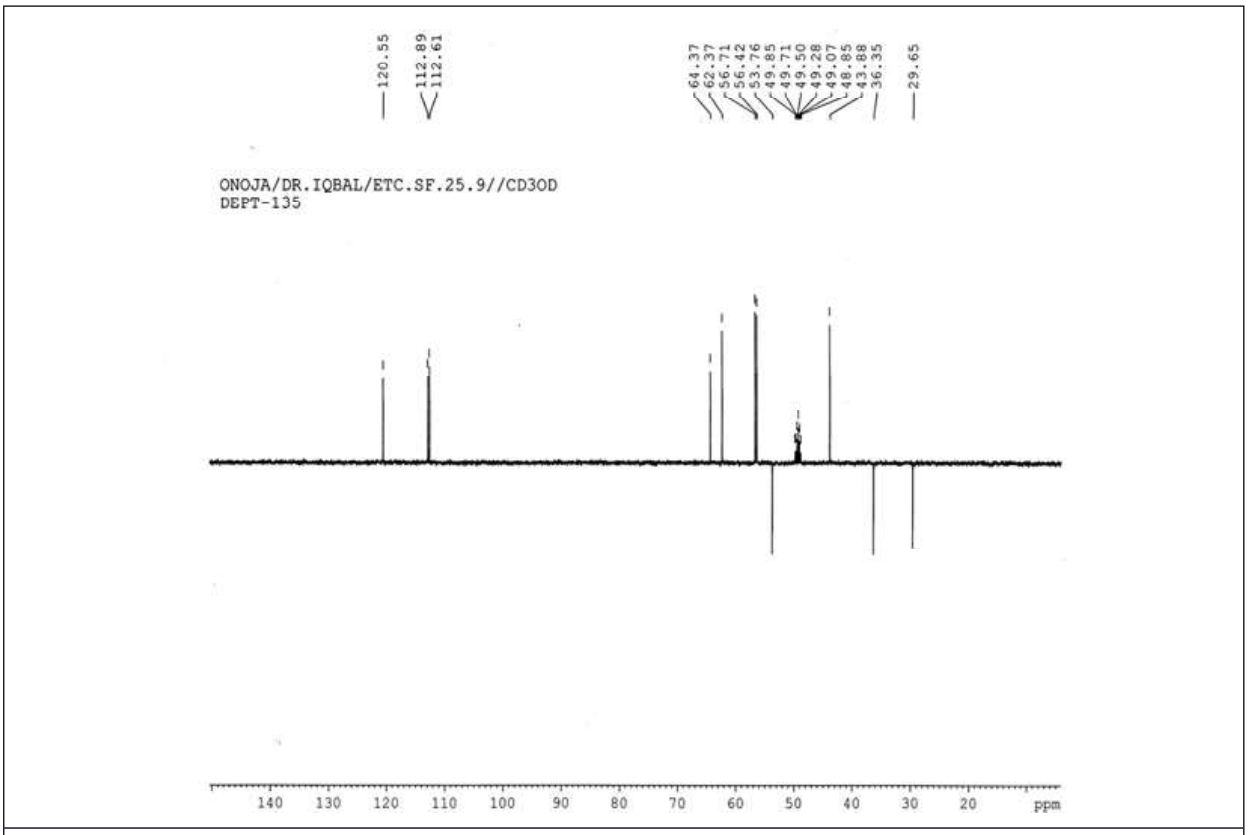


HMBC Spectra of Corydine

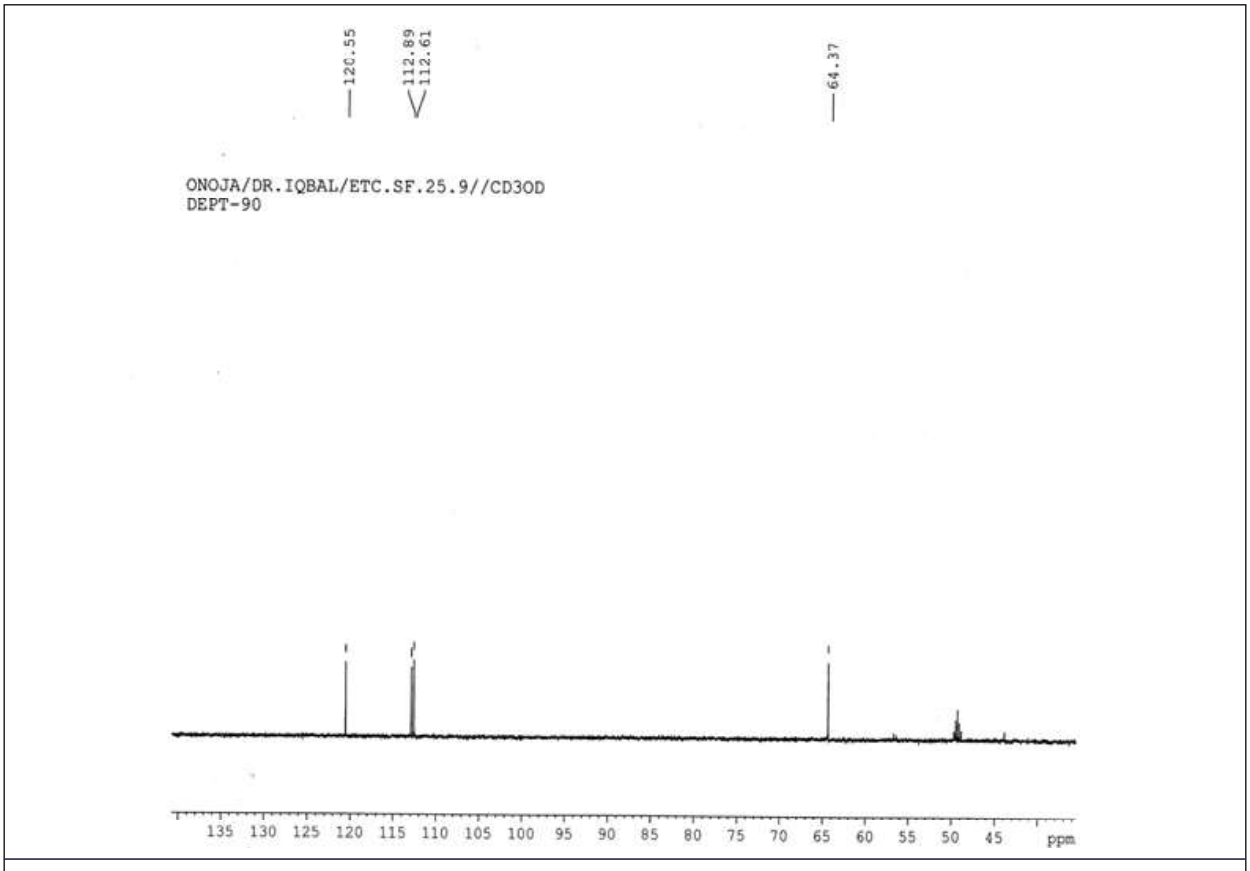


BB Spectra of Corydine

Appendix 1 (Cont.)



DEPT 135 Spectra of Corydine



DEPT 90 Spectra of Corydine

Abbreviations

ROS	:	Reactive oxygen species;
DPPH	:	1,1-diphenyl-2-picrylhydrazyl;
EI-MS	:	Electron Impact Mass spectrometry;
^{1}H-NMR	:	Proton Nuclear Magnetic Resonance;
^{13}C-NMR	:	Carbon-13 Nuclear Magnetic Resonance;
FT-IR	:	Fourier transform infrared spectroscopy;
UV	:	Ultra violet spectroscopy;
EGCG	:	(-)-epigallocatechin-3-gallate;
RSA	:	Radical scavenging activity.

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