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# Hepatoprotective Activities and Bioactive Constituents of *Stephania abyssinica*

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***Authors' contributions***

*Authors APW and DF have made equal contribution to the work. Both authors have read and approved the revised manuscript submitted.*

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## ABSTRACT

**Aims:** To evaluate the hepatoprotective activity of *Stephania abyssinica* (*S. abyssinica*) and isolate the bioactive constituents.
**Study Design:** The *in vivo* hepatoprotective activity of the extracts was evaluated by measuring serum levels of biochemical parameters of liver function in carbon tetrachloride ($CCl_4$) treated rats.
**Place and Duration of Study:** A preliminary survey was conducted in the rural areas of Wolaita (Southern part of Ethiopia) from September to December 2015. Pharmacological and phytochemical studies were conducted at the Laboratories of Department of Pharmacology, and Department of Chemistry of Dilla University.
**Methodology:** The *in vivo* hepatoprotective activity of methanolic extracts of roots and rhizomes of the plant was studied by determining serum levels of the biochemical markers of liver function including glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase (ALP), total protein, and bilirubin concentrations before and after treatments with $CCl_4$ and plant extracts. Bioactive principles were isolated by fractionation followed by concentration by preparative Thin Layer Chromatography. The structures of the compounds were elucidated using spectroscopic techniques including $^{1}H$ NMR, $^{13}C$NMR, DEPT-135, $^{1}H$-$^{1}H$ COSY, HMQC and HMBC.

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**Results:** Administration of $CCl_4$ alone to the rats significantly increased the total bilirubin concentration, GOT, GPT, and ALP (*P = 0.05*). However, simultaneous injection of oral dose of 200 mg/kg b.wt of the extracts reduced the serum levels of the enzymes by 53.42, 33.62, and 55.00% respectively. When 100 mg/Kg b.wt standard drug Silymarin was used, the decrease in the enzyme levels was by 44.00, 66.84, and 58.36%. Our fractionation experiments led to the identification of new hasubanan type alkaloid named as 6-(3'-hydroxy-4'-methoxycinnamoyl) stephine and the containing fraction displayed moderate hepatoprotective activity.
**Conclusions:** The observed hepatoprotective activities of methanolic extracts of roots and rhizomes of *S. abyssinica* lend credence to its claimed traditional use against liver diseases.

*Keywords: 6-(3'-hydroxy-4'-methoxycinnamoyl)stephine; Stephania abyssinica; hepatoprotective, antioxidant*.

## 1. INTRODUCTION

Plants continue to be successful sources of therapeutic agents and attract the attention of researchers and pharmaceutical companies. They owe their success, unlike synthetic drugs, to their accessibility, affordability and ability to address the cause of many diseases and yield superior clinical results. Numerous plants including *Silybum marianum* (Milk thistle), *Picrorrhiza kurroa* (Kutkin), *Curcuma longa* (Turmeric), *Camellia sinensis* (Green tea), *Chelidonium majus* (greater celandine), *Glycyrrhiza glabra* (Liquorice), *Allium sativa* (Garlic), *Andrographis paniculata*, *Phyllanthus niruri* and *Eclipta alba* have been reported to possess hepatoprotective activity [1-4].

*Stephania abyssinica (S. abyssinica)* Walp. (Menispermaceae), indigenous to Southern and Eastern Africa, is one of such plants that has been reported to possess a variety of medicinal uses. According to the indigenous community, an aqueous extract of the roots and rhizomes of the plant is orally taken to treat liver diseases (vernacular name: "Wulawushia) in human. Due to its powerful healing effects, the plant is often accoladed as "medicine of grand disease (Wogga hargiya: Wolaytan)". Previous studies on the plant material revealed hasubanan and aporphine-type alkaloids as principle phytochemical constituents in *S. abyssinica* [5,6] and therapeutic effects including antimalarial [7], antiretroviral [8], and anticancer [9] activities. However, very limited works have been reported regarding the hepatoprotective and antioxidant activities of *S. abyssinica* and its bioactive constituents [10].

This ethnopharmacological and phytochemical investigation on the roots and rhizomes of *S. abyssinica* is conducted to evaluate the hepatoprotective activities of the plant extract vis-à-vis the claimed use against liver diseases and isolate the bioactive constituents. The methanolic extract of the roots and rhizomes of the plant exhibited significant hepatoprotective activity. Several attempts to isolate pure alkaloids as bioactive principles resulted in loss of bioactivity. However, our further fractionation experiments followed by concentration by preparative TLC led to the identification of new hasubanan type alkaloid named as 6-(3'-hydroxy-4'-methoxycinnamoyl)stephine (**1**). The observed hepatoprotective activities of the plant extracts lend credence to the claimed traditional uses of the plant against liver diseases.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The roots and rhizomes of *S. abyssinica* were collected from southern Ethiopia-Boditti (340 km south of the capital, Addis Ababa) in October 2014. The botanical identification of the plant was done at the National Herbarium at Addis Ababa University where a voucher specimen was kept under the cipher ALEM.P1.

### 2.2 Ethnobotanical Study

A survey was conducted in rural areas of different ecological zones during the month of September to December 2015. Ethical guidelines by the International Society of Ethnobiology Code of Ethics [11] were followed during data collection. Written permission was obtained from respective zonal administrators for approaching the community to obtain the indigenous knowledge. In this study, 80 traditional healers (37 men and 43 women) from the age of 18 to 60 were purposively selected from each Zone based on their knowledge and practice on medicinal plants. Local administrators and Development

Agents (DAs) participated in the identification of the key informants. A prior informed consent was also obtained verbally from each informant before commencing the interview. Informants were interviewed individually in the local language (Wolaytan). Semi-structured interview questions regarding informants' biographic details followed by information about medicinal plants used in the treatment of liver diseases, plant local names, effectiveness of plant, plant parts used, method of remedy preparation and mode of administration, and preservation was addressed [12]. The homogeneity level among the collected information was tabulated by the Informants' Consensus Factor, ICF [13]. ICF was calculated using the formula, ICF = (Nuc – Ns)/(Nuc – 1), where Nuc = number of use citations, Ns = number of species used for each use citation [14]. The percentage of informants claiming the use of *S. abyssinica* for "Wulawushia" was calculated as fidelity level (FL): FL (%) = (N / Np) × 100 where, Np = number of informants that claim a use of a plant species to treat liver disease; N = number of informants that use the plants as a medicine to treat liver disease [15]. The survey led to the identification of a total of 11 plants out of which *S. abyssinica* was selected for further investigation based on the values of ICF and FL.

## 2.3 Animals

Wistar albino rats (200-250 g) were purchased from the Ethiopian Health and Nutrition Research Institute (EHNRI) and protected under standard laboratory conditions feeding on commercial rodent feeds and tap water *ad libitum*. The animals were acclimatized to laboratory environment for one week before subjecting to experiments.

## 2.4 Ethical Considerations

The study protocol was prepared in accordance with the internationally accepted principles for laboratory animal use and care and reviewed and approved by Institutional Research Ethics Review (RER) Board of Dilla University. All laboratory animal experiments were conducted as per "principles of laboratory animal care" (NIH publication no. 85-23, revised 1985). All experiments have been examined and approved by the university's RER board.

## 2.5 Materials and Chemicals

All chemicals including hydrochloric acid, Dragendorff's reagents, Mayer's reagents, lead acetate, metallic zinc, concentrated hydrochloric acid, ammonia, gelatin, tannic acid, benzene, iron trichloride, tripotassium ferocyanate, petroleum ether, chloroform, $CCl_4$, DMSO, liver function test kits, ketamine, Ascorbic acid, Silymarin, concentrated sulfuric acid, Tween-80, and 1-1 diphenyl -2-picrylhydrozyl (DPPH) were analytical grade and used as purchased from Aldrich. Solutions were prepared using deionized water obtained with a Milli-Q PLUS (Millipore Corporation). Silica gel 60 (70-230 mesh) was used for column chromatography. TLC was performed on Kieselgel 60 $F_{254}$ (0.20 mm) plates (E. Merck), and the spots were visualized under UV light (254 and 366 nm) and by spraying with Dragendorff's spray reagent followed by heating. Preparative TLC was performed on Kieselgel $HF_{254}$ (0.75 mm thickness).

## 2.6 Apparatus and Equipment

Melting points were measured on a Leica Galen thermo III melting point microscope apparatus and are uncorrected. UV spectra were recorded on a GENESYS 2 PC (200-1100 nm) spectrophotometer in methanol solution. EI-MS was recorded on Finnigan SSQ7000 Spectrometer. *Specific rotations* were recorded on a Perkin-Elmer 241 polarimeter. IR spectra of the samples on KBr pellets were recorded on a Perkin-Elmer Bx (400-4000 $cm^{-1}$) spectrophotometer. NMR spectra were recorded in $CDCl_3$ on Bruker avance 400 MHz (for $^1H$) spectrometer with TMS as internal standard, and chemical shifts are quoted in δ (ppm).

## 2.7 Preparation of the Crude Extract

The dried and powdered roots and rhizomes of *S. abyssinica* were extracted with MeOH at room temperature maceration with occasional stirring for 3 days. The extract was then suction filtered using WHATMAN No.1 filter paper and concentrated at 45℃ under vacuum to oily residue.

## 2.8 Phytochemical Screening

Phytochemical screening of the methanolic root extracts of *S. abyssinica* was carried out using standard phytochemical methods [16].

## 2.9 Acute Toxicity Study

The Wistar albino rats (200-250 g) were divided into five groups of six rats each and subjected to

series of different concentrations (250, 500, 1000, 1500 and 2000 mg/kg of animal b. wt.) of the MeOH extracts of the roots and rhizomes of *S. abyssinica* dissolved in 0.1 ml of DMSO and completed to suitable volume with sterile saline. The extract was orally administered. Then the animals were observed for physical signs of toxicity and subsequent mortality for 14 days.

## 2.10 Screening of Hepatoprotective Activity

The hepatoprotective effect of the extracts was studied by measuring serum GOT, GPT, ALP and total protein and bilirubin concentrations following the procedure previously described [17,18] with slight modification. The animals were divided into four groups of six rats each and fasted for 24 h prior to carbon tetrachloride treatment. The carbon tetrachloride was diluted with paraffin oil at 1:1 proportion. Group I received only 0.9% normal saline 5 ml/kg animal b. wt. as negative group. Group II (positive control) received carbon tetrachloride at a dose of 1.5 ml/kg. Group III received carbon tetrachloride and one hour later 200 mg/kg of animal b. wt. of methanolic root extract for five consecutive days (test group). Group IV animals received carbon tetrachloride and one hour later Silymarin (100 mg/kg) for five consecutive days. All administrations were per oral. During the period of drug treatment the rats were maintained under normal diet and water *ad libitum*. Then on day six animals were anaesthetized with ketamine (100 mg/Kg b.w., im.) and the blood sample of each animal was collected separately by cardiac puncture into sterilized dry centrifuge tubes and allowed to coagulate for 30 min. Serum was separated by centrifugation at 3000 rpm for 15 min. The serum was used to estimate biochemical markers. These biochemical parameters were assayed spectrophotometrically using a commercially available assay kits.

## 2.11 *In vitro* DPPH Assay

The free radical scavenging activity of the plant extracts was determined with 1, 1- diphenyl-2-picrylhydrazyl (DPPH) assay. The method reported by [19] was employed with some modifications. To 3 ml solution of 0.004% DPPH (in methanol), 0.1 ml of various concentrations (1000, 500, 250, 125, 50, µg/ml) of the crude extracts or ascorbic acid (a reference compound) were added. Then after 30 min incubation at room temperature, the absorbance was read against a blank at 517 nm using Unico TM2100 spectrophotometer. Each sample was measured in triplicate and averaged. The percentage of radical scavenging activity was calculated using the following formula:

$$\%\ radical\ scavenging = \frac{A_0 - A_1}{A_0} \times 100$$

Where $A_0$ is the absorbance of the control (3 ml DPPH solution in methanol) and $A_1$ is the absorbance of the sample extracts. Lower absorbance value indicates higher free radical scavenging activity. The 50% inhibitory concentration value ($IC_{50}$) is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals.

## 2.12 Bioactive Constituents and Structure Elucidation

Alkaloids were selected as target compounds in this study based on the fact that a great number of alkaloids originating from plant origin have already showed a potent activity against parasitic protozoa and several helminthes as natural medicines or provided lead structures for novel synthetic drugs [20,21]. The crude methanol extract obtained from the above procedure (section 2.7) was partitioned between chloroform and 5% HCl. The acidified aqueous layer was separated from the organic phase, separated from an insoluble tar by filtration, basified with excess ammonia solution and extracted three times with chloroform (total of 500 ml). The resultant crude basic chloroform extract yielded fraction 10 g residue upon evaporation under vacuum. 5 g of the residue was subjected to silica gel column chromatography [CC], eluent: 5% MeOH in $CHCl_3$, to give 80 fractions. Fractions nos. 25 – 40 were combined ($F_1$) and further purified by silica gel [CC] followed by preparative TLC to give known compounds. Fractions nos. 55 – 75 which were eluted with increasing polarity, were combined (**$F_2$**) and further purified by repeated preparative TLC to give compound **1** (50 mg). Structure elucidation of the isolated compound was performed using spectroscopic techniques including IR, $^{1}H$ (400MHz), $^{13}C$ (100 MHz) and correlated NMR spectra (DEPT-135, $^{1}H$-$^{1}H$ COSY, HMQC and HMBC) and mass spectrometry.

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical Screening

Our phytochemical investigation has revealed the presence of alkaloids, flavonoids, saponins, and phenolic compounds in methanolic extracts of the roots and rhizomes of *S. abyssinica*. Tannins, coumarins, and anthraquinones were found to be absent in the extract. The presence of alkaloids which are common natural therapeutic agents and such constituents as flavonoids and phenolic compounds, which usually display antioxidant properties, in the roots and rhizomes extracts of *S. abyssinica* served as a preliminary indication of the hepatoprotective and antioxidant potential of *S. abyssinica*.

### 3.2 Acute Toxicity Study

Toxicity study was carried out to determine the safety of the plant and select safe dose range to be used for the experiment. The animals showed good tolerance to the testing doses as high as 1000 mg/kg b.w. p.o. However, at doses between 1000 and 2000 mg/kg b. wt a dose dependent behavioral sensitivities such as convulsive movements were observed. Thus, 200 mg/kg b. wt was selected as testing dose for further experiments. The dose dependent behavioral sensitivity is consistent with the ethnomedicinal information suggested by the local community and previous report [8]. Thus 1/10th of the sensitivity dose (200 mg/kg b.wt) was chosen as test doses.

### 3.3 Hepatoprotectivity

In order to validate the claimed use of the plant against liver diseases, hepatoprotective activity of the plant extracts was evaluated based on its effect on serum biochemical parameters following $CCl_4$ induced oxidative damage to liver in rats [22]. As indicated in Table 2 animals treated with toxic dose of carbon tetrachloride had markedly elevated levels of the serum hepatic enzymes and reduced level of protein and increased level of total bilirubin. The level of enzyme markers GOT, GPT, and ALP in normal rats were found to be 265.3±13.5, 101.2±1.1, 245.0±7.8 IU/L respectively; as expected, the carbon tetrachloride intoxication made their elevation to 1.18, 2.57 and 1.71-fold increment with the values of 578.8±6.4, 361.3±15.7 and 664.3±16.1 IU/L respectively (Table 1). Treatment with the extracts significantly ($P < 0.05$) reduced their elevations by 53.42, 33.62, and 55. 00% with the values of 270±12.4, 239.8±5.6, and 299.3±7.9 IU/L for GOT, GPT and ALP respectively. The corresponding decreases in GOT, GPT and ALP levels after treatment with Silymarin were 44.00, 66.84, and 58.36 % with the values of 324.5, 119.8, and 276.6 respectively. The extract had also reversed the bilirubin elevation and protein depletion by factors of 1.90 and 1.70 respectively.

**Table 1. Effects of methanolic extracts of roots and rhizomes of *S. abyssinica* on liver functions of carbon tetrachloride ($CCl_4$) intoxicated rats**

| Groups (n = 5) | Treatment | Liver function markers | | | | |
|---|---|---|---|---|---|---|
| | | GOT[a] | GPT[b] | ALP[c] | Total bilirubin (g/dl) | Total protein (g/dl) |
| I | Normal control | 265.3±13.5a | 101.2±1.1a | 245.0±7.8a | 7.4±0.10 | 69.12±0.43 |
| II | 50 % CCl4 control (1.5 ml/kg b. wt.) | 578.8±6.4 | 361.3±15.7 | 664.3±16.1 | 23.8±0.13 | 37.9±0.17 |
| III | $CCl_4$ + root extract (200 mg/kg b. wt.) | 270±12.4a | 239.8±5.6a | 299.3±7.9a | 12.50±0.01 | 64.13±0.50 |
| IV | $F_2$ | 504.13 | 316.23 | 549.69 | 17.83 | 54.11 |
| V | $CCl_4$+ Silymarin (100 mg/kg b. wt.) | 324.5±22.4a | 119.8±3.1a | 276.6±3.9a | 9.83±0.02 | 57.95±0.08 |

*F2: fractions from column chromatography containing compound 1; [a]Glutamate oxaloacetate transaminase, [b]Glutamate pyruvate transaminase, [c]Alkaline phosphatase (ALP)*

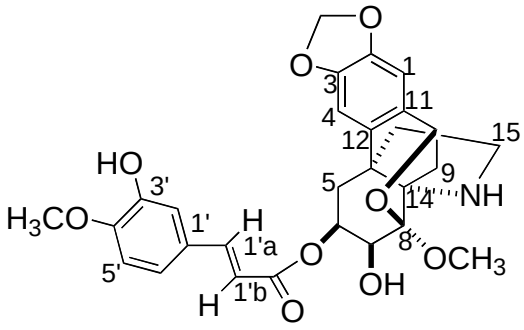
When rats are treated with $CCl_4$, it induces hepatotoxicity by metabolic activation involving cytochrome $P_{450}$ dependent mixed oxidase in the endoplasmic reticulum to form trichloromethyl free radical which combines with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation [23,24]. These result in loss of structural integrity and increase in serum activities of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and alkaline phosphatase (ALP). While the elevation in serum activities of the enzymes following the intoxication by $CCl_4$ might be due to the hepatic injury and loss of structural integrity, a decrease in their level and that of bilirubin following treatment with root extracts suggest stabilization of plasma membrane as well as repair of hepatic tissue damage caused by the $CCl_4$. The reversal in protein concentration towards increment also suggested a healthy function of the liver as a result of recovery by the extract.

### 3.4 *In vitro* Antioxidant Activity

Flavonoids and phenolic compounds are common antioxidants. Since such class of compounds are present in the extract of *S. abyssinica* as confirmed by our phytochemical screening, and that oxidative stress to cells following injection of lethal dose of $CCl_4$ is one of the possible causes for hepatic injury, it was important to validate the observed hepatoprotective activity of the plant extract in terms of its antioxidant properties. Thus, the antioxidant activity of the methanolic extracts of *S. abyssinica* roots was assayed by using DPPH radical. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The results from in vitro antioxidant activity test showed that the methanolic root extracts of the *S. abyssinica* and ascorbic acid (standard antioxidant) display dose dependent free radical scavenging activity with an IC50 of 3.11 µg/ml and 2.92 µg/ml respectively. The dose dependent free radical scavenging activity of *S. abyssinica* reinforce the hypothesis that one of possible mechanism for the hepatoprotective activity of the root extract by free radical scavenging.

### 3.5 Bioactive Constituents

Although several attempts to isolate the bioactive constituent(s) resulted in significant loss of bioactivity, our fractionation experiments followed by concentration by preparative TLC *led to* the identification of new hasubanan type alkaloid named as 6-(3'-hydroxy-4'-methoxycinnamoyl) stephine (Fig. 1). The characterization of the compound was based on $^1$H (400MHz), $^{13}$C (100 MHz) and correlated NMR spectra and mass spectrometry. Complete $^1$H and $^{13}$C-NMR chemical shifts along with $^1$H-$^1$HCOSY and HMBC correlations for the two alkaloids isolated in this work are given in Tables 2.




**(1)**

**Fig. 1. Structures of the new compound, 6-(3'-hydroxy-4'-methoxycinnamoyl) stephine (1) isolated from methanolic extracts of roots and rhizomes of *S. abyssinica***

**6-(3'-hydroxy-4'-methoxycinnamoyl) stephine (1):** was isolated as a white solid and crystallized from MeOH to give white needles; mp 270-277℃. $[\alpha]^D$ = +29 (c = 0.85, MeOH) optical act], UV $\lambda_{max}$(A); 236.0 nm (4.000), 282.0 nm (3.750) and 323.0 nm (3.322). Its molecular formula was determined to be $C_{28}H_{29}NO_9$ as deduced from its EI-MS and NMR spectroscopic analysis. The EI-MS showed a molecular ion at m/z 523.1842 (calc. 523.1837) consistent with the molecular formula. The IR spectrum exhibited a broad absorption at 3366 $cm^{-1}$ due to hydroxyls. Among other absorptions are the carbonyl (C=O) stretch at 1702 $cm^{-1}$ and out-of-plane alkene C-H bending vibrations at (985-810 $cm^{-1}$). Other IR absorptions included 3020, 3008, 2932, 2870, 1634, 1612, 1581, 1510, 1485, 1441, 1376, 1260, 1129, 1110, 1038, 985, 931, 858, 811, 761, 581 $cm^{-1}$.

The $^1$H-NMR of **1** (Table 2) showed five aromatic protons: two singlets ($\delta_H$6.45 *s*, 6.55 *s,* 2H), two

doublets ($\delta_H$ 6.75 *d* (J=8.2 Hz), 6.92 *d* (J=1.6 Hz), 2H) and one doublet of doublets ($\delta_H$ 6.85 *d* (J=1.6, 8.2 Hz), 1H) indicating the aromatic substitution pattern. It also showed two methoxy groups ($\delta_H$3.50*s* and 3.82*s*) and two clearly distinguished vinylic protons [$\delta_H$ 5.45 *d* (J=16 Hz), 7.05 *d* (J=16 Hz)] whose signals appeared as sharp doublets pointing to *trans* coupling. The difference in their chemical shifts is significant ($\Delta\delta$ = 1.60 ppm) and diagnostic of an $\alpha$, $\beta$ unsaturated carbonyl group with aryl substituent at the $\beta$ position. The $^{13}$C-NMR and DEPT-135 spectra revealed the presence of five methylene carbons (including the methylenedioxy carbon), ten methine carbons, eleven quaternary and two methoxy carbons. The assignments of the protonated carbons and their C-C connections (C5-C6-C7, C9-C10, C15-C-16 and C1'a-C1'b) were performed by application of $^{1}$H-$^{1}$H COSY and HSQC experiments whereas their attachment to the quaternary carbons were based on HMBC correlations (Fig. 2).

The high-field shifts ($\delta_H$ 5.00, 5.57) and the splitting pattern of the methylenedioxy protons (d, *J* =1.4 Hz) indicated the close proximity of the aromatic ring of the cinnamoyl moiety to the methylenedioxy protons. Additionally, the value of the coupling constants (*J*= 2.53Hz, 3.20 Hz) between the C-5 methylene protons and the C-6 proton were indicative of either axial-equatorial or equatorial-equatorial coupling and not of diaxial

**Table 2. $^{1}$H-NMR, $^{13}$C- NMR, $^{1}$H-$^{1}$H COSY, and HMBC Data for 1 in $CDCl_3$[a]**

| Position | $^{\delta}$H[b] | $^{\delta}$C[c] | $^{1}$H-$^{1}$H COSY | HMBC ($^{1}$H→$^{13}$C) Correlation |
|---|---|---|---|---|
| 1 | 6.55*s* | 106.5 | | 77.4,146.2,148.3137.2,107.7 |
| 2 | | 146.2 | | |
| 3 | | 148.3 | | |
| 4 | 6.45*s* | 107.7 | | 47.5,106.5,133.5,148.3,146.2 |
| 5 | 2.32*m* (J=3.20Hz,16.0Hz) 1.85*m* (J=2.53Hz,16.0Hz) | 36.1 | 1.85,4.95 2.32,4.95 | 47.5,133.5,73.1,102.3,72.2 |
| 6 | 4.95 *m* | 73.1 | 2.32,2.15,4.02 | |
| 7 | 4.02 *d* (4.40) | 72.2 | 4.95 | 102.3 |
| 8 | | 102.3 | | |
| 9 | 2.35 *dd* (6.00, 11.2), 2.15 *d* (11.2) | 39.3 | 2.15,4.75 2.35 | 73.4,77.4,137.2 |
| 10 | 4.75 *d* (5.60) | 77.4 | 2.35 | 73.4,106.5,102.3,137.2 |
| 11 | | 137.2 | | |
| 12 | | 133.5 | | |
| 13 | | 47.5 | | |
| 14 | | 73.4 | | |
| 15 | 3.05 *m* | 41.6 | 1.96 | 39.3 |
| 16 | 1.96 *m* | 39.3 | 3.05 | 73.4,36.1,47.5 |
| 1' | | 128.4 | | |
| 2' | 6.92 *d* (1.60) | 113.5 | 6.85 | 121.9,148.8,145.5 |
| 3' | | 145.5 | | |
| 4' | | 148.8 | | |
| 5' | 6.75 *d* (8.20) | 110.9 | | 148.8,128.4,145.5 |
| 6' | 6.85 *dd*(1.60, 8.20) | 121.9 | | 110.8,148.8 |
| 1'a | 7.05 *d* (16.0) | 144.1 | | 166.9,115.9,128.4,113.5,121.9 |
| 1'b | 5.45 *d* (16.0) | 115.9 | | 166.9,128.4 |
| 8-OMe | 3.50 *s* | 52.4 | | 102.3 |
| 4'-OMe | 3.82 *s* | 56.4 | | 148.8 |
| -$OCH_2O$- | 5.00 d (1.4) | 101.3 | 5.57 | 148.3 |
| | 5.57 *d* (1.4) | | 5.00 | 146.2 |
| Ester C=O | | 166.9 | | |

[a] Chemical shifts are in ppm from internal TMS [b] at 400 MHz; *J* values in Hz are given in parentheses [c] at 100 MHz; chemical shifts in ppm

coupling. These findings suggested that the cinnamoyl moiety attached to C-6 has β-axial and the OH on C-7 has β-equatorial orientations [5,9,25]. Complete NMR data were summarized as indicated in Table 1. The above findings and comparison of the NMR data of **1** with those of closely related known compounds such as stephavanine, suggested **1** to be 8,10-epoxy,7-hydroxy-8-methoxy-2,3-[methylenebis(oxy)]has ubanan-6-[3'-hydroxy-4'-methoxycinnamate] or 6-(3'-hydroxy-4'-methoxycinnamoyl) stephine.

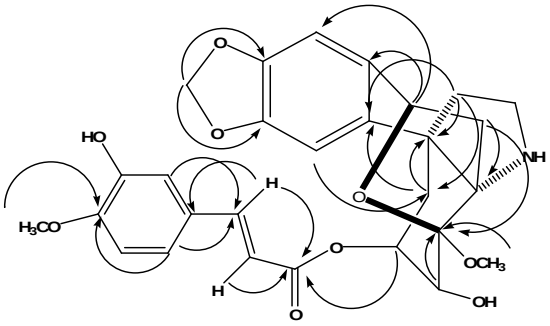



**Fig. 2. HMBC correlations detected for 6-(3'-hydroxy-4'-methoxycinnamoyl) stephine isolated from *S. abyssinica***

The observed hepatoprotective and antioxidant activities of the root extracts of *S. abyssinica* is further evidenced by isolation of bioactive constituents. The assignments of protons and carbons of the isolated compounds were consistent with the characteristic spectral properties of the aporphine-like structure 2 [26]. It is interesting to note the difference between the $^{1}$H-NMR chemical shifts of the methylene protons of the methylenedioxy group of 2 and that of 1. The difference in the chemical shifts for the methylene protons of the methylenedioxy group of 2 is 0.2 ppm, whereas that of 1 is significant ($\Delta\delta$ = 0.59 ppm) and diagnostic of the axial (β) orientation of the cinnamoyl moiety discussed above.

## 4. CONCLUSIONS

The methanolic extracts of the roots and rhizomes of *S. abyssinica* exhibited significant hepatoprotective activities. The observed hepatoprotective and antioxidant activities of the methanolic activities of the roots and rhizomes of the plant and the matching structure of the isolated compound- [6-(3'-hydroxy-4'-methoxycinnamoyl)] lend credence to the claimed traditional uses of the plant against liver diseases. The study further indicated that the plant might be of value as therapeutic agent if proper screening against cytotoxic constituents, which seem to be responsible for the observed side effects of the plant, is made.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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