

## Synthesis, identification and anticonvulsant activity of dehydrozingerone

Enas M. Awad<sup>1\*</sup>, Elhadi M.M Ahmed<sup>2</sup>, Tarig M. Hashim El-hadiyah<sup>3</sup>, Nizar Sirag<sup>2</sup> and Mohammed Abdelrahman<sup>4</sup>

- 1- Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Gezira, Sudan.
- 2- Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Sudan.
- 3- Unit of Pharmacology and Therapeutics, School of Pharmacy, Ahfad University for Women, Sudan.
- 4- Department of Pharmaceutics, Faculty of Pharmacy, University of Gezira, Sudan.

\* Correspondence: [enasmohamed@uofg.edu.sd](mailto:enasmohamed@uofg.edu.sd) Tel. 00249511842726

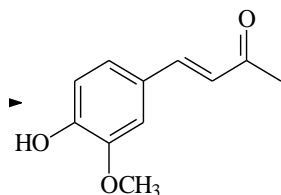
### ABSTRACT

The present study aimed to synthesize and characterize dehydrozingerone **as well** as to investigate its anticonvulsant activity in experimental animals. A simple method was used to synthesize dehydrozingerone using vanillin and acetone. The synthesized drug dehydrozingerone was characterized using thin layer chromatography (TLC), high performance liquid chromatography (HPLC), infrared spectroscopy (IR) and physicochemical tests. The synthesized product was tested for its potential anticonvulsant activity using maximum electroshock (MES) induced seizure models in rats. The synthesized dehydrozingerone showed TLC profile, FT IR spectra and HPLC chromatogram similar to the authentic sample. The physicochemical characters (colour, taste, flavour, solubility and melting point) were also similar to what was found in the literature. All these results indicated that the produced product was dehydrozingerone. A dose dependent anticonvulsant activity was produced by dehydrozingerone. Eighty percent anti-MES activity was presented by 100 mg/kg. The findings indicated that dehydrozingerone represents a bioactive molecule possessing anticonvulsant activity. In addition, it is an easily synthesized compound from cheap starting materials. In conclusion dehydrozingerone may find its place as antiepileptic agent if further clinical studies will be conducted.

**Key words:** Dehydrozingerone, synthesis, anticonvulsant activity.

## INTRODUCTION

Dehydrozingerone is a pungent constituent present in the rhizomes of ginger (*Zingiber officinale*) and belongs structurally to the vanillyl ketone class (Yogosawa *et al.*, 2012). Dehydrozingerone is reported to possess many biological activities such as: antioxidant (Kuo *et al.*, 2005), antitumor (Motohashi *et al.*, 1998), inhibitory effect on vascular smooth muscle cell function (Yizhen *et al.*, 2008) and antifungal (Kubra *et al.*, 2012) activities. The present study aimed to synthesize and characterize dehydrozingerone as well as to investigate its anticonvulsant activity in experimental animals.



### Dehydrozingerone

Product	category	Aromatic ketone
:		
Molecular	formula	C <sub>11</sub> H <sub>12</sub> O <sub>3</sub>
:		
Molecular	weight	192.2
:		
Melting	point	125-130 °C
:		
Boiling	point	348.2 °C
:		
Synonyms		Dehydrozingerone
:		Dehydro[0]-paradol
		Feruloylmethane
		3-Methoxy-4-hydroxybenzalacetone
		4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one
		4-hydroxy-3-methoxystyrylmethylketone

## MATERIALS AND METHODS

### MATERIALS

#### Chemicals:

Pentylentetrazole, dehydrozingerone and vanillin were purchased from Sigma-Aldrich, UK, acetone, hydrochloric acid, ethanol, sodium hydroxide and tween<sub>20</sub> were obtained from Loba Chemie, India.

### METHODS

#### Preparation of working solutions of chemicals:

Freshly prepared solutions of pentylentetrazole dissolved in normal saline and dehydrozingerone dissolved in 5% tween<sub>20</sub> were used.

#### Experimental animals:

Albino rats of both sexes weighing 150 – 200 g were used. The animals were kept and maintained under appropriate laboratory conditions, allowed free access to water and fasted for an over night before the experiment.

#### Synthesis of dehydrozingerone:

Vanillin (4g) was placed in a beaker. Acetone (20 ml) and 10% sodium hydroxide solution (20 ml) were added to vanillin. The mixture was stirred, stoppered and allowed to stand for 48 hours. After standing, the dark colored mixture is acidified with 10% hydrochloric acid (60 ml), placed in an ice bath and stirred. Upon acidification and stirring a yellowish-brown solid was formed. The solid material was filtered and washed several times with cold water. The crude product was recrystallized from 50% aqueous ethanol (Allen *et al.*, 2006).

#### Identification of dehydrozingerone:

#### Melting point determination:

The melting point of the synthetic dehydrozingerone was determined in sealed capillary tubes on an electrothermal melting point apparatus (Veego Scientific MP-D. India). Results were the mean of triplicate readings.

#### Thin layer chromatography (TLC) for synthetic dehydrozingerone:

The synthesized dehydrozingerone and the standard reference were chromatographed on TLC sheet, silica gel 60 of thickness 0.2mm (Riedel-de Haen AG. Seelze Germany) using toluene and ethyl acetate (93: 7) as

solvent system. Iodine vapour was used as locating agent.  $R_f$  values were calculated.

### **Infrared spectroscopy (FT IR) for synthetic dehydrozingerone:**

IR spectra of both synthesized and reference dehydrozingerone were recorded on FTIR spectrophotometer-8400 S (Shimadzu-Japan), using KBr disk of IR spectroscopic grade (Shimadzu Laboratory reagents, Japan).

### **High performance liquid chromatography (HPLC) for synthetic dehydrozingerone:**

The synthesized dehydrozingerone and the standard reference were analyzed by high performance liquid chromatography (Waters, USA) equipped with Shim-pack VP-ODS column C18 (250\* 4.6 mm). Isocratic elution was performed using a mixture of HPLC grade methanol and water (65:35 v/v), flow rate 1.0ml/min, temperature 28°C. Photodiode Array Detector was set at 280nm. Sample injection volume was 20  $\mu$ l.

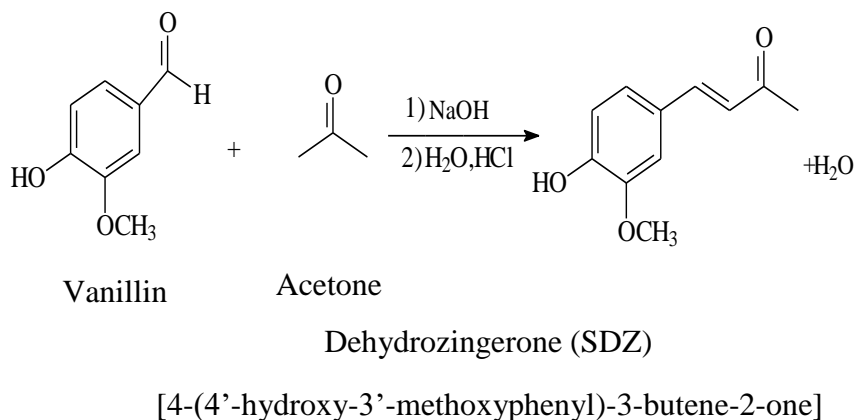
### **Maximal electroshock-induced seizure test:**

Maximal electroshock (MES) seizure model was used to evaluate the anticonvulsant activity of dehydrozingerone. Seizures were induced in rats by delivering electroshock of 50 mA for 0.5 second by means of an electro-convulsimeter (Ugobasile ECT unit 57800) through a pair of ear clip electrodes (Kumar *et al.*, 2008). Six groups of rats of both sexes were used. Three groups of rats (n=5) received dehydrozingerone (10, 50 and 100 mg/kg) intraperitoneally (i.p) (Manigauha *et al.*, 2009). Thirty minutes later rats were tested for MES induced seizure response. Two groups of rats (n=3) received sodium valproate (300 and 400 mg/kg) injected i.p. and tested after 15 minutes for MES induced seizure response. All the experimental groups were compared to a control group administered the vehicle (tween<sub>20</sub>).

## RESULTS AND DISCUSSION

### Synthesis and identification of dehydrozingerone (DZ):

Literature reports showed an easy method for dehydrozingerone (C<sub>11</sub>H<sub>12</sub>O<sub>3</sub>) synthesis, in which two starting materials, vanillin and acetone were used (Allen *et al.*, 2006). .



### Fig. 1: Synthesis of dehydrozingerone.

The reactions produced fragrant, brilliant yellow powder of good yield (1.99 g; 50%). The powder is soluble in alcohol and insoluble in water. Melting point was determined as being 126-127 °C. Results obtained agree with what has been reported by Allen *et al.*, (2006). The conducted DZ synthetic method produced a considerable yield of the product in lower number of steps. TLC profiles (Fig. 2), HPLC chromatograms (Fig. 3) and FT IR spectra (Fig. 4) of the synthesized dehydrozingerone (SDZ) are almost matching with those obtained from dehydrozingerone (DZ) standard sample. For TLC, identical R<sub>f</sub> value (0.2) was observed using iodine vapour as locating agent whereas HPLC analysis revealed similar retention time (1.32 minute).

FT IR spectra represents bands at 3303 (broad band), 3001, 2948, 2848, 2362, 1676, 1637, 1581, 1517, 1452, 1425, 1367, 1298, 1263, 1224, 1166, 1122, 1024, 979, 937, 875, 821, 804, 756, 671, 576, 543, and 464  $\text{cm}^{-1}$  .

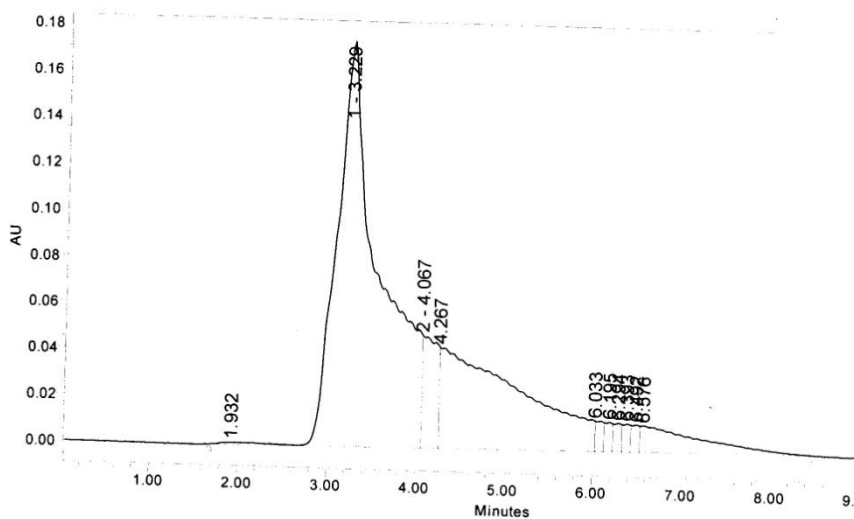
The characteristic and diagnostic bands of dehydrozingerone are visible in the region (3303-1581  $\text{cm}^{-1}$ ). All the literature (Hatzade *et al.*, 2009; Hatzade *et al.*, 2008; Dudley and Fleming, 1980) agrees in assigning a band at 3303  $\text{cm}^{-1}$  to the stretching of O-H bond. Two characteristic aryl C-H stretching bands also appeared at 3001  $\text{cm}^{-1}$  and 2362  $\text{cm}^{-1}$ . Aliphatic C-H stretching appeared at 2948 and 2848  $\text{cm}^{-1}$ . Carbonyl conjugated double bond stretching was represented by a band at 1676  $\text{cm}^{-1}$ . Aromatic C=C stretching appeared at 1637 $\text{cm}^{-1}$  and 1581 $\text{cm}^{-1}$ . In addition the overcrowded lower IR fingerprint region of dehydrozingerone, where many absorption bands occur was found to be identical to the reference sample, presenting band at 1224  $\text{cm}^{-1}$  for phenolic O-H bending and aryl alkyl ether of characteristic two peaks; asymmetric C-O-C stretch at 1263  $\text{cm}^{-1}$  and a symmetric stretch at 1024 $\text{cm}^{-1}$ .

The analytical data obtained and physicochemical characters (colour, taste, flavour, solubility and melting point), TLC profile, FT IR spectrum and HPLC chromatogram for the SDZ coincide with those of the reference sample as well as they match with data shown in the literature confirming that the synthesized compound was indeed dehydrozingerone .

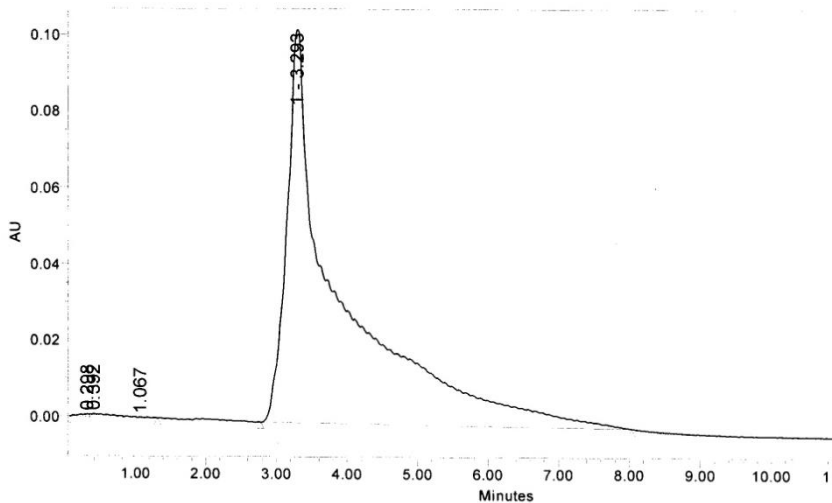


**Fig.2: TLC chromatogram of (a): synthesized dehydrozingerone  
(b): reference dehydrozingerone.**

A)

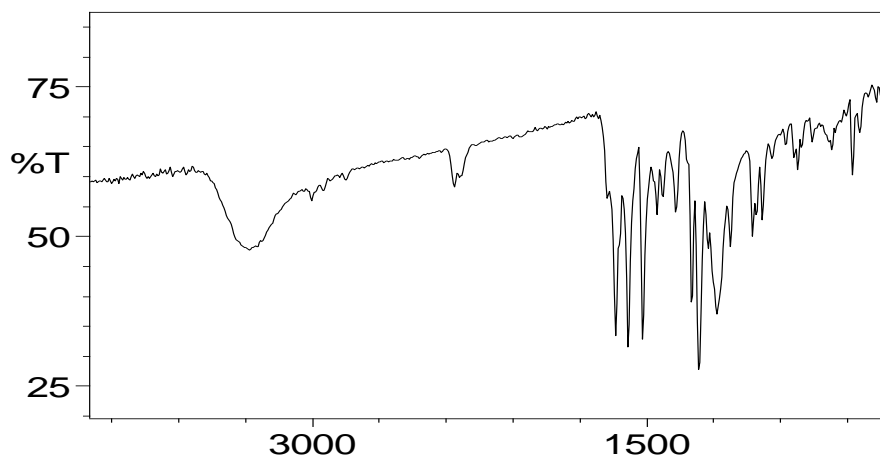


B)

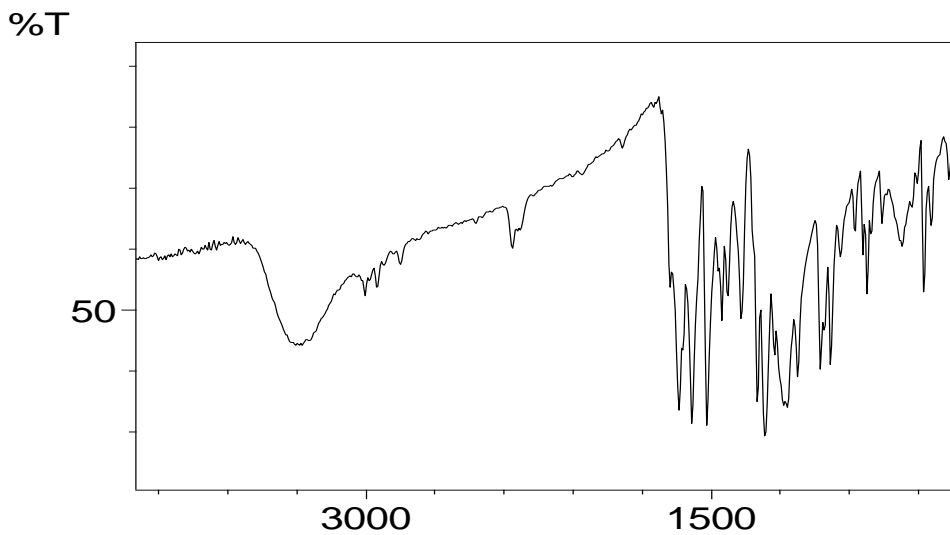


**Fig. 3: HPLC chromatogram of (A) Reference dehydrozingerone (B) Synthetic dehydrozingerone.**

**A)**



**B)**



**Fig.4: FTIR spectra of (A) Reference dehydrozingerone (B) synthetic dehydrozingerone.**

**Anticonvulsant activity of the synthesized dehydrozingerone:**

As shown in Table 1 dehydrozingerone produced a dose dependent anticonvulsant activity in the maximum electroshocks seizure model used. High doses were required to prevent seizure induced by MES. Eighty percent anti-MES activity was presented by 100mg/kg. There was no previous reported data about the anticonvulsant activity of dehydrozingerone. Dehydrozingerone presents a reactive molecule containing vanillyl aromatic moiety, conjugated double bond system, reactive carbonyl group and capability of hydrogen bonding, all that may contribute to its anticonvulsant activity.

**Table 1: Anti-maximum electroshocks (MES) activity of the synthetic dehydrozingerone .**

Treatment	Vehicle	Sodium valporoate (standard)		Dehydrozingerone		
		300	400	10	50	100
Dose mg/kg	10ml/kg	300	400	10	50	100
Seizure protection (%)	0.00	66.6	100	20	40	80

**CONCLUSIONS**

The findings indicated that dehydrozingerone represents a bioactive molecule possessing anticonvulsant activity. In addition, it is an easily synthesized compound from cheap starting materials. In conclusion dehydrozingerone may find its place as antiepileptic agent if further clinical studies are conducted.

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