

Full Length Research paper

# Isolation and characterization of a new chalcone from the leaves of *Heteropyxis natalensis*

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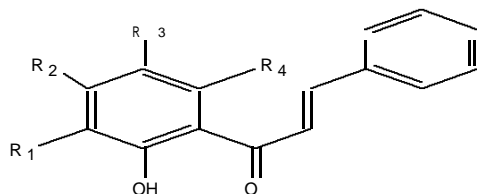
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Chromatographic analysis of the defatted dichloromethane extract of the leaves of *Heteropyxis natalensis* afforded the isolation of (*E*)-1-(2',4'-dihydroxy, 5'-methoxy, 3'-methylphenyl)-3-phenylprop-2-en-1-one (R<sub>1</sub> = Me; R<sub>2</sub> = OH; R<sub>3</sub> = OMe; R<sub>4</sub> = H) a chalcone. The structure of this compound was established by its spectroscopic data-1D, 2D-NMR, EIMS and HR-EIMS.

**Key words:** Chalcones, *Heteropyxis natalensis*, heteropyxidaceae, flavone.

## INTRODUCTION



[1]: R<sub>1</sub> = Me; R<sub>2</sub> = OH; R<sub>3</sub> = OMe; R<sub>4</sub> = H

[2]: R<sub>1</sub> = H; R<sub>2</sub> = OH; R<sub>3</sub> = Me; R<sub>4</sub> = OMe

[3]: R<sub>1</sub> = Me; R<sub>2</sub> = OMe; R<sub>3</sub> = H; R<sub>4</sub> = OH

*Heteropyxis natalensis* Harvey (Heteropyxidaceae) commonly known, as lavender tree is a small well-foliated deciduous tree that grows to about 10 meters high (Palgrave, 1977). It occurs naturally on the coastal and inland regions of KwaZulu-Natal province of Southern Africa. Heteropyxidaceae is a small family with only three species known in Southern Africa namely: *Heteropyxis canescens*, *H. dehniae* and *H. natalensis*.

*H. natalensis* is used among the Zulus as medicinal tea Its bark is used to treat impotence and as an aphrodisiac. Nose bleeding, is checked by inhaling the steam from a decoction of the roots. The leaves are reputedly used to scent tobacco and dosed to stock in powdered form, to eradicate intestinal worms (Hutchings et al., 1996)

Previous investigators have examined the essential oils from the leaves of *H. natalensis* (Sibanda et al., 2004; Muzuru Gundidza et al., 2006). The antioxidant activity of the phenolic constituent of *H. natalensis* of Zimbabwe has been determined (Mchuweti et al., 2006). There is no report on the non-volatile phytochemicals from this plant in literature. Our search for non-volatile bioactive natural products as leads for new ethnopharmaceuticals, led us to examine the leaves of *H. natalensis* for its phytochemical potentials.

## MATERIALS AND METHODS

### Experimental

Melting points were determined on a Stuart Scientific SMPI apparatus, IR spectra (KBr) were recorded on a Nicolet Impact 420 spectrophotometer, NMR spectra (both 1D and 2D) were obtained on a Varian 300 (300 MHz) spectrometer, using the residual solvent peaks as internal standards, HR-EIMS were determined on a Kratos 9/50 instrument. Column chromatography was carried out using Merck Silica gel 60 (70 – 230 mesh). Analytical TLC was carried out on precoated aluminium plates using Merck Silica gel F254; plates were visualized under UV light (λ 254 and 366 nm) and by spraying with anisaldehyde/H<sub>2</sub>SO<sub>4</sub> reagent, followed by gentle heating.

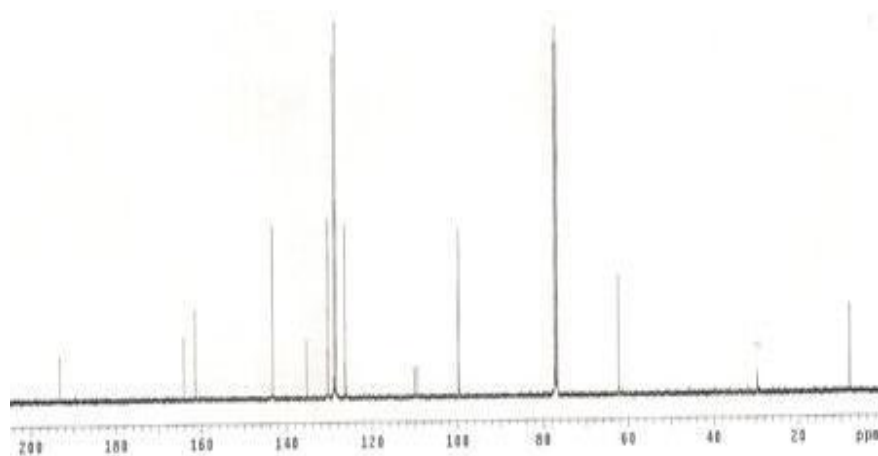
### Plant material

Fresh leaves of *H. natalensis* were collected in October 2004 from Durban and identified by H. Baijnath. A voucher specimen (MH/06) was deposited in the Wards Herbarium, UKZN- Westville Campus.

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**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR of ( $\text{R}_1 = \text{Me}$ ;  $\text{R}_2 = \text{OH}$ ;  $\text{R}_3 = \text{OMe}$ ;  $\text{R}_4 = \text{H}$ ).

Position	C-atom	$\delta^{13}\text{C}$ (ppm)	$\delta^1\text{H}$ (ppm), J (Hz)	HMBC
1	C	135.28		
2	CH	128.45	7.64 – 7.62 m	
3	CH	128.95	7.41 – 7.38 m	
4	CH	130.30		
5	CH	128.95		
6	CH	128.45	7.64 – 7.62 m	
$\alpha$ C	CH	126.38	7.82 d, 15.6	193.17, 135.28, 143.27
$\beta$ C	CH	143.27	7.95 d, 15.6	193.17, 135.28, 126.38, 128.45
1'	C	109.55		
2'	C	161.51		
3'	C	110.25		
4'	C	164.13		
5'	C	161.45		
6'	CH	99.73	6.22 s	164.13, 161.45, 109.55
C=O	C	193.17		
OMe	$\text{CH}_3$	62.24	3.67 s	161.45
Me	$\text{CH}_3$	8.04	2.11 s	161.51, 110.25, 109.55
OH			13.28s	

**Figure 1.**  $^{13}\text{C}$ -NMR of ( $\text{R}_1 = \text{Me}$ ;  $\text{R}_2 = \text{OH}$ ;  $\text{R}_3 = \text{OMe}$ ;  $\text{R}_4 = \text{H}$ ).

### Extraction and isolation

The powdered air-dried leaves of *H. natalensis* (1.1 kg) was successively extracted by maceration at room temperature in hexane, dichloromethane, ethyl acetate and methanol to give, after removal of solvent *in-vacuo*, hexane extract (19.1g), DCM extract (39.6 g), ethyl acetate extract (16.5 g) and methanol extract (126.5 g), respectively.

Column Chromatography of the dichloromethane extract (3 g) on silica gel, by gradient elution with hexane and hexane/ethylacetate mixtures followed by purification of fractions gave a yellowish crystalline compound (105 mg), melting point: 139 - 140°C.

IR (KBr):  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3252 (br OH), 3106, 3048, 1631, 1505, 1413.

$^1\text{H}$  and  $^{13}\text{C}$  nmr: Table 1 and Figure 1 and 2

EIMS:  $m/z$  (rel. int. %, Fig 5) = 284  $[\text{M}]^+$  (40), 267 $[\text{M-OH}]^+$ (05), 207  $[\text{M-C}_6\text{H}_5]^+$  (20), 180  $[\text{M-C}_6\text{H}_5\text{CH=CH}_2]^+$  (100), 152  $[\text{180-C=O}]^+$  (45).

HREIMS:  $m/z$   $[\text{M}^+$ , 100] 284.1041 (Cal. 284.1049 for  $\text{C}_{17}\text{H}_{16}\text{O}_4$ ).

## RESULTS AND DISCUSSION

Extraction of dried pulverized and defatted leaves of *H.*

*natalensis* (1.1 kg) with dichloromethane afforded 39.6 g extract. Open column chromatographic fractionation of the extract (3.0 g) and purification of fractions led to isolation of  $\text{R}_1 = \text{Me}$ ;  $\text{R}_2 = \text{OH}$ ;  $\text{R}_3 = \text{OMe}$ ;  $\text{R}_4 = \text{H}$ , a yellowish crystalline solid (105 mg) mp 139 - 140°C.

The  $^{13}\text{C}$  nmr spectrum (Table 1) of solid  $\text{R}_1 = \text{Me}$ ;  $\text{R}_2 = \text{OH}$ ;  $\text{R}_3 = \text{OMe}$ ;  $\text{R}_4 = \text{H}$  showed 17 signals including two CH signals at  $\delta$ 126.38 and  $\delta$ 143.27 corresponding to the  $\alpha$  and  $\beta$  carbons of chalcones (Pelter et al., 1976); six aromatic CH signals at  $\delta$ 130.30, 128.95 (2C), 128.45 (2C) and 99.73; six quaternary carbons at  $\delta$ 164.13, 161.51, 161.45, 135.28, 110.25 and 109.55; two  $\text{sp}^3$  carbons at  $\delta$ 62.24 (OCH<sub>3</sub>) and  $\delta$ 8.04 (CH<sub>3</sub>) and a carbonyl carbon at  $\delta$ 193.17.

Its  $^1\text{H}$  nmr showed a downfield signal at  $\delta$ 13.28 due to a chelated hydroxyl (this was confirmed by ir spectrum with a broad OH band at  $3252\text{ cm}^{-1}$  and a chelated carbonyl band at  $1631\text{ cm}^{-1}$ ), 2Hm aromatic ( $\delta$ 7.63), 3Hm

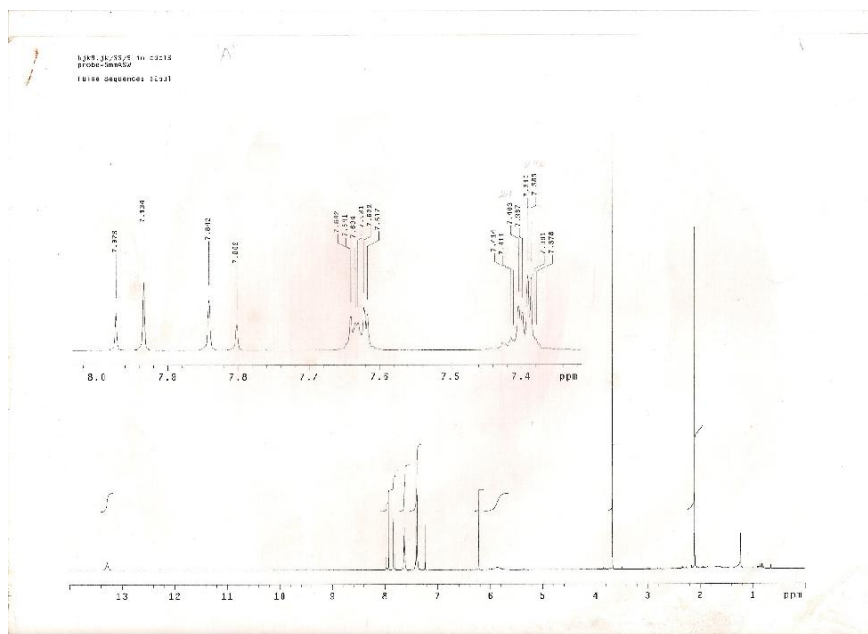


Figure 2. <sup>1</sup>H-NMR Spectrum of (R1 = Me; R2 = OH; R3 = OMe; R4 = H).

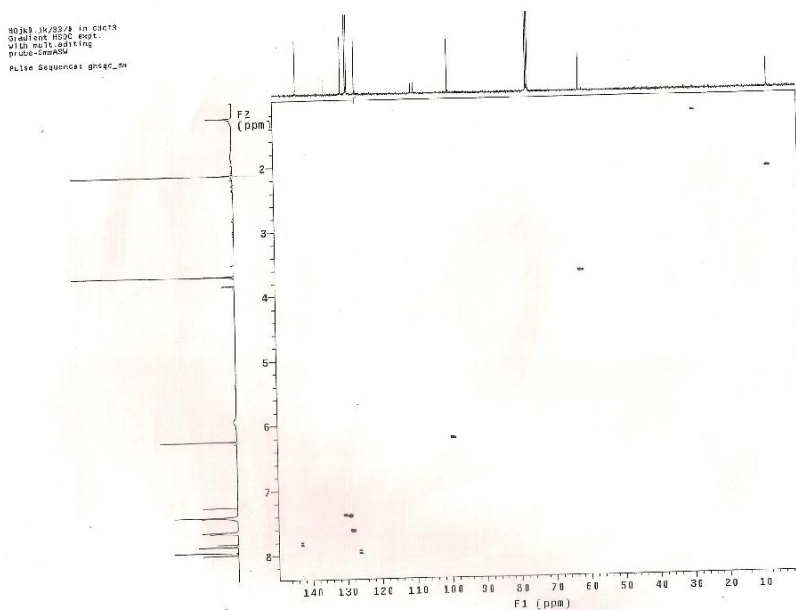


Figure 3. HSQC Spectrum of (R1 = Me; R2 = OH; R3 = OMe; R4 = H).

aromatic ( $\delta$  7.40), two doublets at  $\delta$ 7.96 (J 15.6Hz) and  $\delta$ 7.82 (J 15.6Hz), one aromatic singlet at  $\delta$ 6.22, and two other singlets at  $\delta$ 3.67 (methoxy group) and  $\delta$ 2.11 (methyl group). These data are indicative of a 2'-hydro-xylyated chalcone. Compound R<sub>1</sub> = Me; R<sub>2</sub> = OH; R<sub>3</sub> = OMe; R<sub>4</sub> = H is an isomer of aurentiacin A R<sub>1</sub> = H; R<sub>2</sub> = OH; R<sub>3</sub> = Me; R<sub>4</sub> = OMe and triangularin R<sub>1</sub> = Me; R<sub>2</sub> = OMe; R<sub>3</sub>

= H; R<sub>4</sub> = H, chalcones isolated from *Myrica serrata* (Stefan et al., 1996) and *Pityrogramma triangularis* (Star et al., 1978) respectively.

Proton/Carbon Correlations- HSQC and HMBC spectra (Figure 3 and 4): The HSQC spectrum gave clear unambiguous correlations of the carbon atoms with the protons directly attached to them (Table 1) In the HMBC

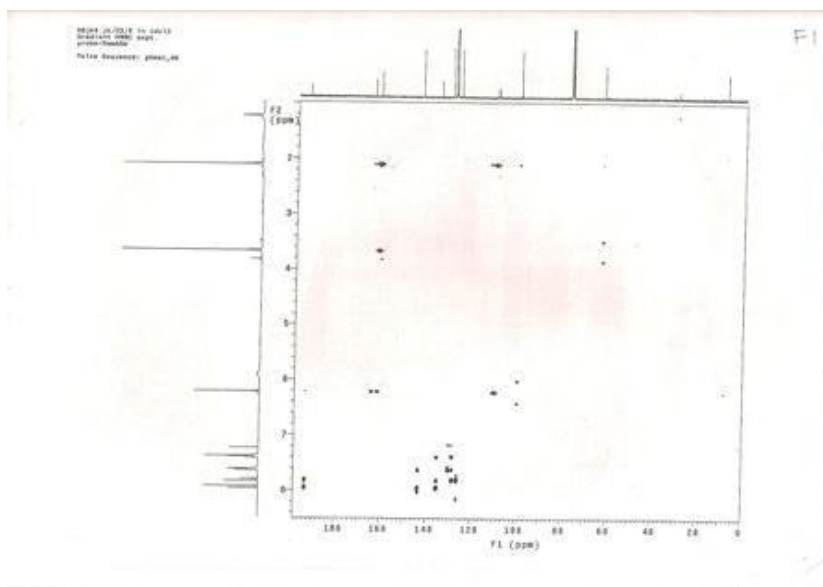


Figure 4. HMBC Spectrum of (R1 = Me; R2 = OH; R3 = OMe; R4 = H).

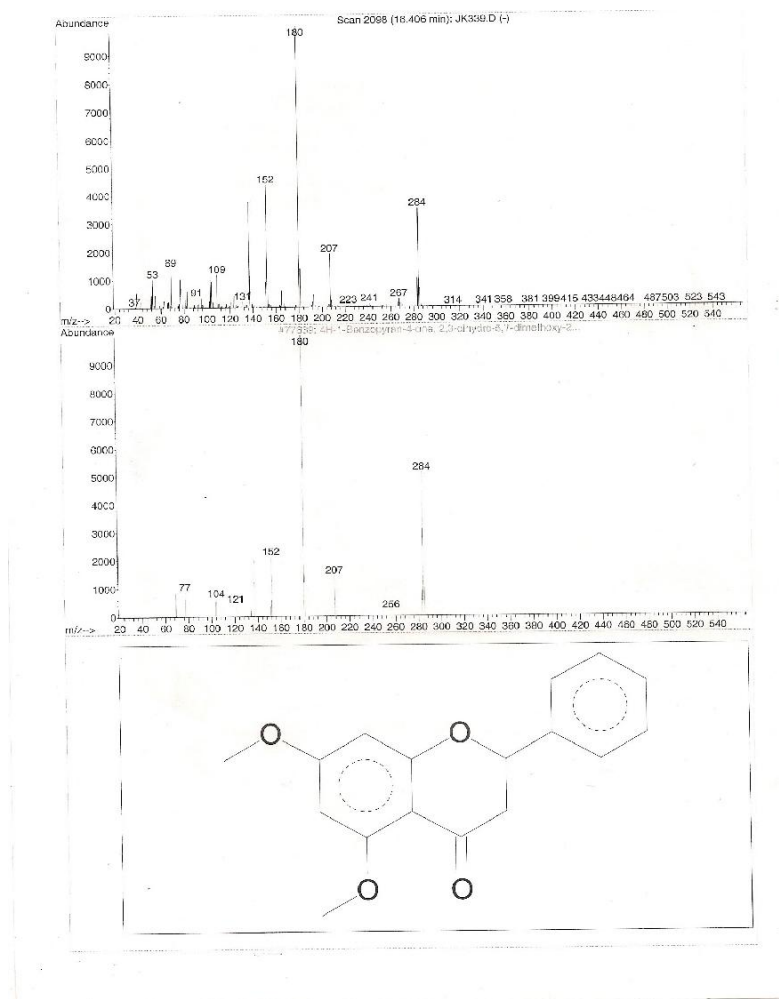
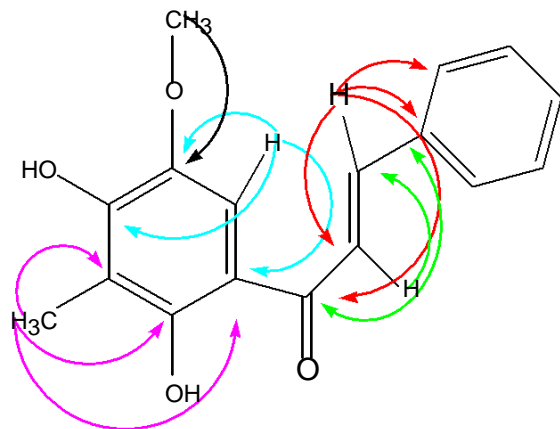


Figure 5. EIMS Spectrum of (R1 = Me; R2 = OH; R3 = OMe; R4 = H).



**Figure 6.** HMBC Correlations in (R1 = Me; R2 = OH; R3 = OMe; R4 = H).

spectrum, the position of the methoxy group at 5' was confirmed by the correlation of the methoxy protons at 3.67 ppm with carbon C-5'. The methyl signal at C -3' was similarly situated with observed correlations of the methyl protons with C-2', C-3', C-4' and C-1' (Table 1, Figure 6).

The base peak fragment ion in the EIMS ( $m/z$  180) corresponds to the carbonyl -bond cleavage giving the chealation- / resonance-stabilized ion.

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