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RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF CATECHIN AND CATECHIN-3-O-GALLATE FROM THE BARK OF Vitellaria paradoxa (SAPOTACEAE)

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Abstract

Background: The emergence of drug-resistant microorganisms has led to the screening of medicinal plants for alternative. *Vitellaria paradoxa* of Sapotaceae family, is a medicinal plant of vast use in traditional medicine for the treatment of many diseases including cancer, bacterial and fungal infections. The aim of this study is to isolate and characterize bioactive compounds from the ethyl acetate stem bark extract of *V. paradoxa.*
Methods: The powdered stem bark was extracted successively by maceration using n-hexane, ethyl acetate and ethanol. The dichloromethane soluble portion of the ethyl acetate extract (MD) was subjected to column chromatography and fraction (coded MD168) obtained by pooling of fractions based on their TLC profiles was purified and characterized using Liquid Chromatography-Mass Spectrometer (LC-MS), one dimensional (1D) and two dimensional (2D) Nuclear Magnetic Resonance (NMR) techniques including Heteronuclear Single Quantum Coherence (HSQC), Heteronuclear Multiple Bond Correlation (HMBC) and Proton-Proton Correlation Spectroscopy (^{1}H-^{1}H COSY) and by comparison with the literature.
Results: Based on the spectral data obtained, MD168 was identified as a mixture of two known types of flavanol of flavonoid class: catechin and catechin-3-O-gallate.
Conclusion: The two compounds were previously isolated from the other parts and extracts of *V. paradoxa* and were reported to be active against strains which make them potential precursors for new drug synthesis.

Keywords: *Vitellaria paradoxa, catechin, catechin-3-O-gallate, LC-MS, Spectroscopy, NMR, HSQC, HMBC, COSY*

1.0 INTRODUCTION

The side effects of some synthetic drugs (The American Society of Health-System Pharmacists, 2017) and increase in resistant strains have led to the screening for more effective, less toxic and cost-effective drugs (Okpuzor *et al.,* 2021) from natural sources (Bhatt and Neggi, 2012). Plants are the major source of pharmaceuticals, and they provide bioactive compounds that can be used directly as drugs or as synthetic favour of approaches based on combinatorial chemistry and genomics (Gurnani *et al*., 2014). The recent studies suggested that plant products are rich source of many biologically active phenolic compounds which have been found to possess potent antioxidant as well as antimicrobial activity (Kaneria *et al*., 2012). Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl; thus, inhibiting the oxidative mechanisms that lead to degenerative diseases (Subramanion *et al.,* 2011).

Due to the notable pharmacological effects and nutritional values, several researchers have extensively investigated and isolated active constituents of stembark, kernel, leaves, root, and nutshells of *V. paradoxa.* However, the issue emerging drug resistant microorganisms makes it necessary to isolate and characterize more compounds from this plant with the aim of providing alternative drugs to tackle the issue of drug resistance as new compounds are reportedly isolated from the plant in recent studies by Ramsay *et al.* (2016), Eyong *et al.* (2018) Da *et al.* (2019), Sinan *et al.* (2020), Buxton *et al.* (2020) and Maiyama *et al.* (2020)

2.0 METODOLOGY

2.1 Sampling, Preparation of Plant Material and Extraction

The plant sample of *V. paradoxa* was collected at Maiyama town, Maiyama Local Government Area of Kebbi State, Nigeria. The sample was authenticated at the Herbarium Section of the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, Nigeria with voucher number [UDUH/ANS/0114]. The fresh sample was shade dried for two weeks, pulverized into fine powder using mortar and pestle and stored in a plastic container until further use. The extraction was carried out using the method of Tsafe *et al*., (2019) thus: the sample (400 g) was extracted with 2 L of n-hexane (1: 5 w/v) in a closed container by maceration with occasional shaking for five days. The extract obtained was filtered with muslin cloth, Whatmann no. 1 filter paper and concentrated *in vacuo* at 45 ^{0}C using rotary evaporator. The residue was macerated with ethyl acetate and then methanol respectively using the same procedure as described above. All the extracts obtained were stored in different containers and labeled for further analysis. The percentage yield of the extracts was calculated using Equation 1:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times 100 \quad \text{----------1}$$

2.2 Column Chromatography on Ethyl acetate Extract.

The ethyl acetate extract was chosen for isolation of compounds based on its highest antioxidant activity. The reddish dichloromethane (DCM) soluble fraction labelled MD (2.1 g) was fractionated on a silica gel column, thus: A slurry of silica gel (50 g) was dispersed into 200 cm^3 of hexane: ethyl acetate (9:1) and stirred with a glass rod. The slurry was introduced into a glass column (2.3 cm by 45 cm) and the column was run gradiently using 200 cm^3 of 95 % hexane in ethyl acetate to hexane 100 % ethyl acetate (each step in the gradient represented a 5 % increase in polarity). A total of 285 fractions 10 cm^3 each were collected and pooled together based on their TLC profiles. Fractions 160-168 (coded MD168) were selected.

2.3 Purification of MD168

The purification was done using Preparative Thin Layer Chromatography (PTLC). PTLC was carried out using silica gel precoated plates (25x20 cm). A thin line was drawn using pencil at 1.5cm from the bottom of the plate. The sample was then dissolved in a small amount of methanol to yield an approximate concentration of 20mg/ml. It was then uniformly applied along the thin line using

Pasteur glass pipette and allowed to dry after which it was developed using DCM: methanol (9:1) solvent system. The plate was air dried, the position of the band of interest was marked with pencil and scraped off onto a foil and the sorbent was transferred to a sintered glass funnel and repeatedly washed with methanol. The solution obtained was evaporated to give the compound (Gibbons and Gray, 1998).

2.4 Characterization of the MD168

The isolated compound (MD168) was characterized by means of physical tests, chemical tests, spectroscopic methods namely Liquid Chromatography-Mass Spectrometry (LC-MS), NMR spectroscopic analysis (1D and 2D) as well as by comparison with the existing literature.

2.4.1 Physical Tests on MD168

The isolated compound (MD168) was subjected to solubility test using different solvents.

2.4.2 Chemical Tests on MD168

The isolated compound (MD168) was subjected to ferric chloride test by adding three drops of ferric chloride solution to the solution of the compound. Subsequently, dilute hydrochloric acid (HCl) was added (Sofowora, 2008).

2.4.3 Spectroscopic Analysis of MD168

Liquid Chromatography-Mass Spectroscopy of MD168 was conducted using LC-MS machine (model: 6200 series TOF/6500 series Q-TOF 10.1 (48.0), manufacturer: Agilent) and Nuclear Magnetic Resonance (NMR) spectroscopic analysis (1D and 2D) using NMR spectrophotometer (model: Bruker AMX400 spectrometer) using deuterated acetone (acetone$_{-d6}$). Both analyses were carried out at the Department of Organic Chemistry, University of Glasglow, United Kingdom.

3.0 RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 DCM Soluble Extract, TLC Profiles of the Column Fractions

Plate 1 showed the reddish DCM soluble portion of the ethyl acetate extract (MD), its TLC plate and column fractions.

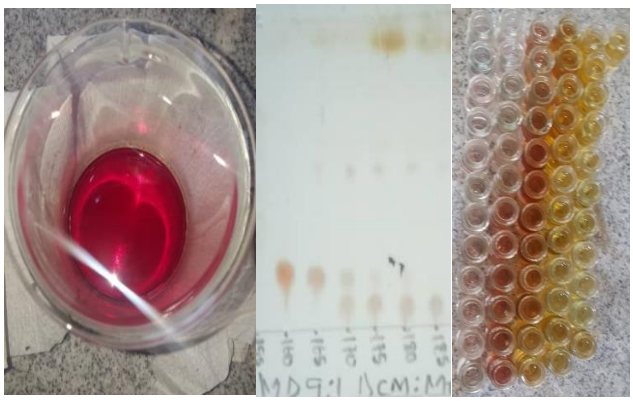


Plate 1: DCM Soluble Extract, TLC Profiles of the Column Fractions

3.1.2 Characterization of MD168

3.1.2.1 Physical Test on MD168 (Appearance and Solubility)

MD168 reddish brown and was soluble in acetone.

3.1.2.2 Chemical Test on MD168

MD168 was found to be positive on ferric chloride test with appearance of greenish color which turned colorless on addition of dilute HCl. (Evans, 2002)

3.1.2.3 LC-MS Spectral Analysis of MD168

Figure 1 shows the LC-MS Spectra of MD168 with molecular ion peak at Mass to Charge Ratio (m/z) 290 and $[M+H]^+$ equal to 291, corresponding to molecular formula of $C_{15}H_{14}O_6$

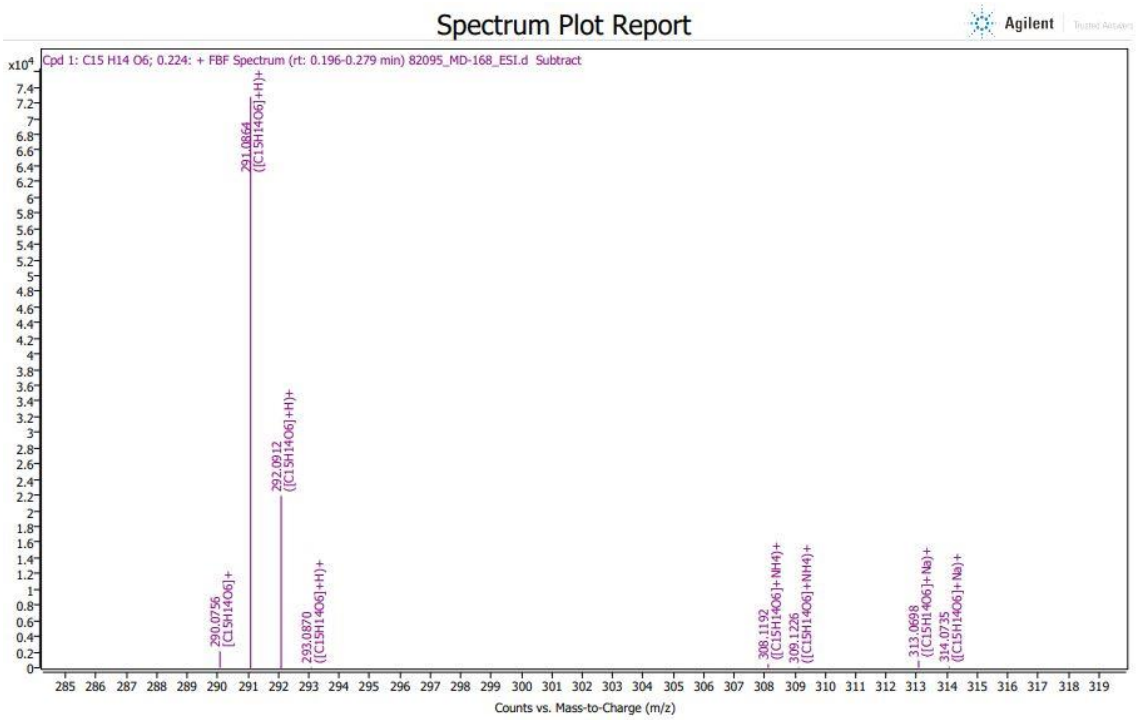
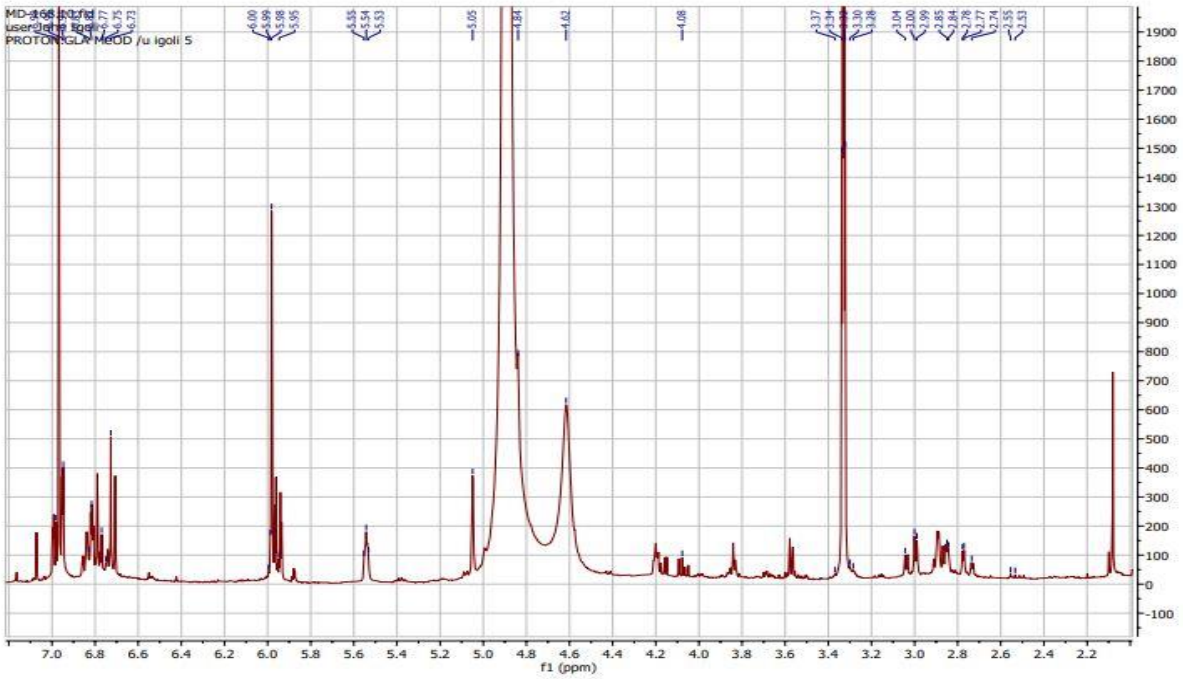


Figure 1: LC-MS Spectrum of MD168

3.1.2.4 Proton Nuclear Magnetic Resonances (^{1}H NMR) spectral analysis of MD168

Figure 2 showed the ^{1}H-NMR spectrum of MD168 showing a range of signals in pairs forming crowded peaks across the spectrum. A pair of doublets of doublets between δ_H 2.7 and 3.0 is also observed.



3.1.2.5 ^{13}C-NMR spectrum MD168

Figure 3 showed ^{13}C-NMR spectrum of ME311, showing peaks at δ_C 78.49 and 77.24 for carbons 2 (C-2), 68.60 and 66.60 (C-3), 27.86 and 25.47 (C-4), 98.70 and 98.01 (C-10), 95.18 and 95.03 (C-6), 94.52 (C-8), 156.46 (C-5), 156.60 (C-7), 156.27 (C-9), 130.89 and 130.06 (C-1'), 113.94 and 113.73 (C-2'), 144.55 (C-3'), 144.92 (C-4'), 114.63 and 114.52 (C-5'), 117.99 (C-6'), 120.08 (C-1''), 108.84 (C-2'' and C-6''), 138.41 (C-4'') and 166.23 (C-O)

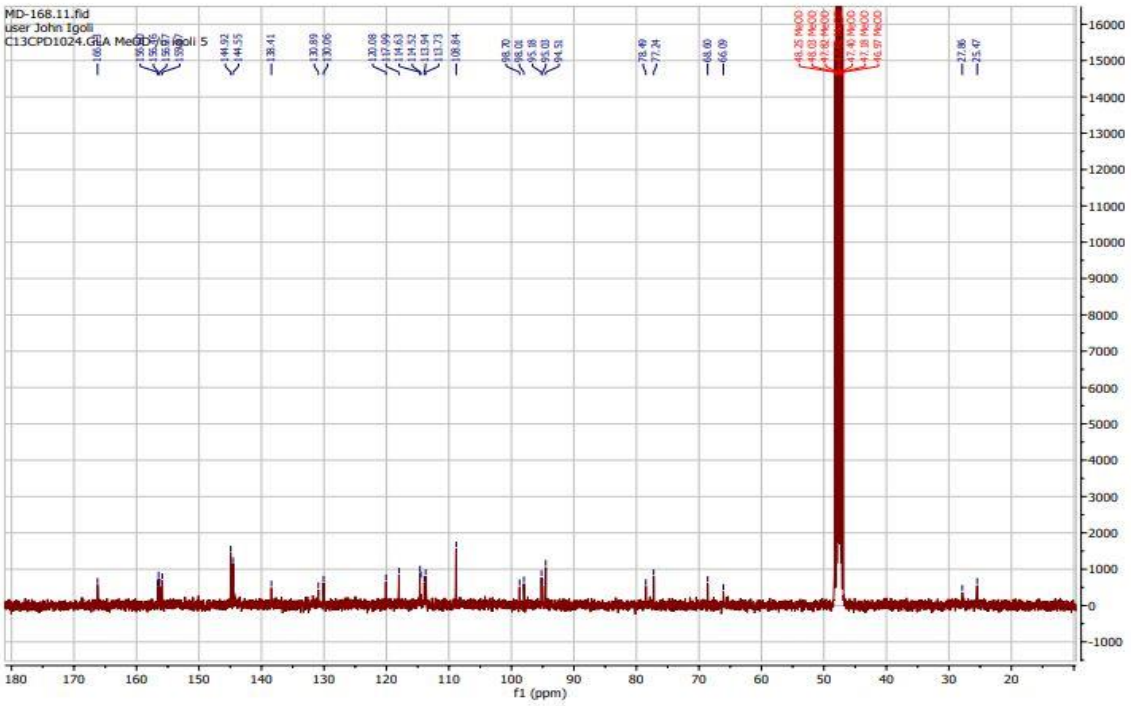


Figure 3: ^{13}C-NMR spectrum MD168

3.1.2.6 HSQC spectrum MD168

Figure 4 showed the spectrum of MD168 from which correlations between carbons and protons attached to them are observed

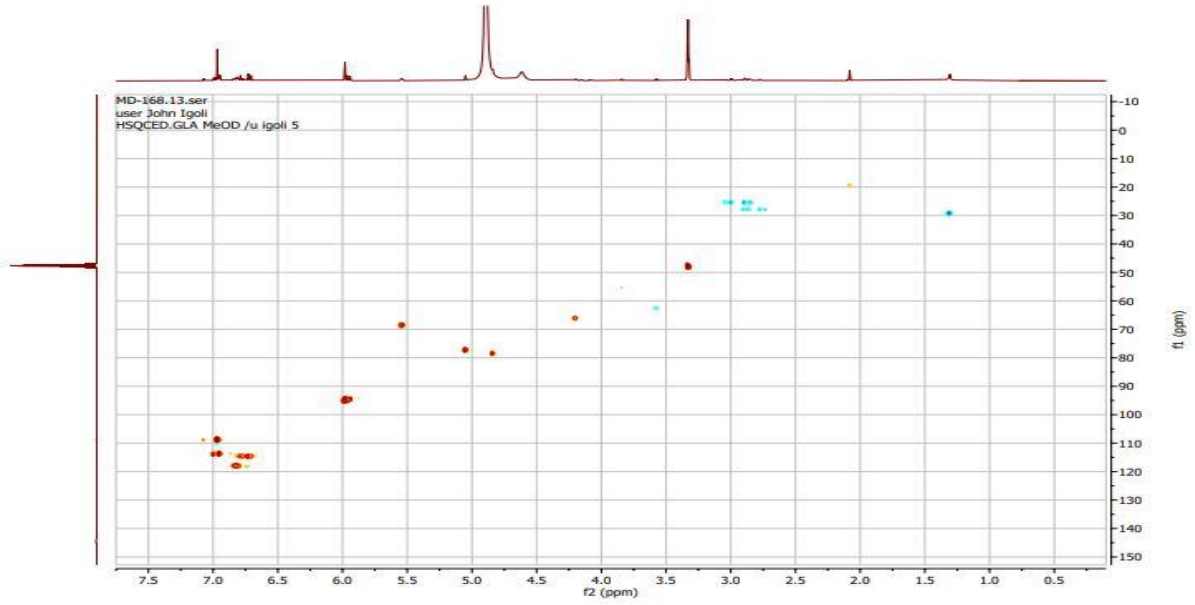


Figure 4: HSQC spectrum MD168

3.1.2.7 HMBC spectrum MD168

Figure 5 showed HMBC spectrum of MD168 where long range correlations between protons and carbons of the compound(s) are observed.

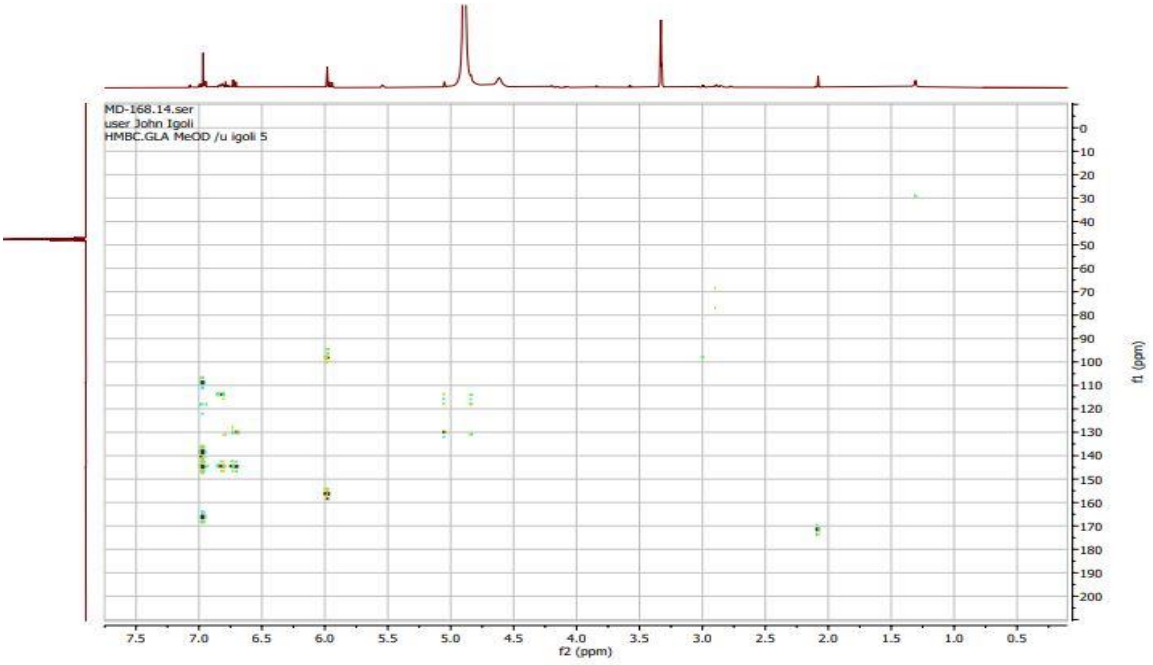


Figure 5: HMBC spectrum MD168

3.1.2.8 COSY spectrum MD168

Figure 6 showed COSY spectrum of MD168 from which correlations between protons of the compound(s) are observed.

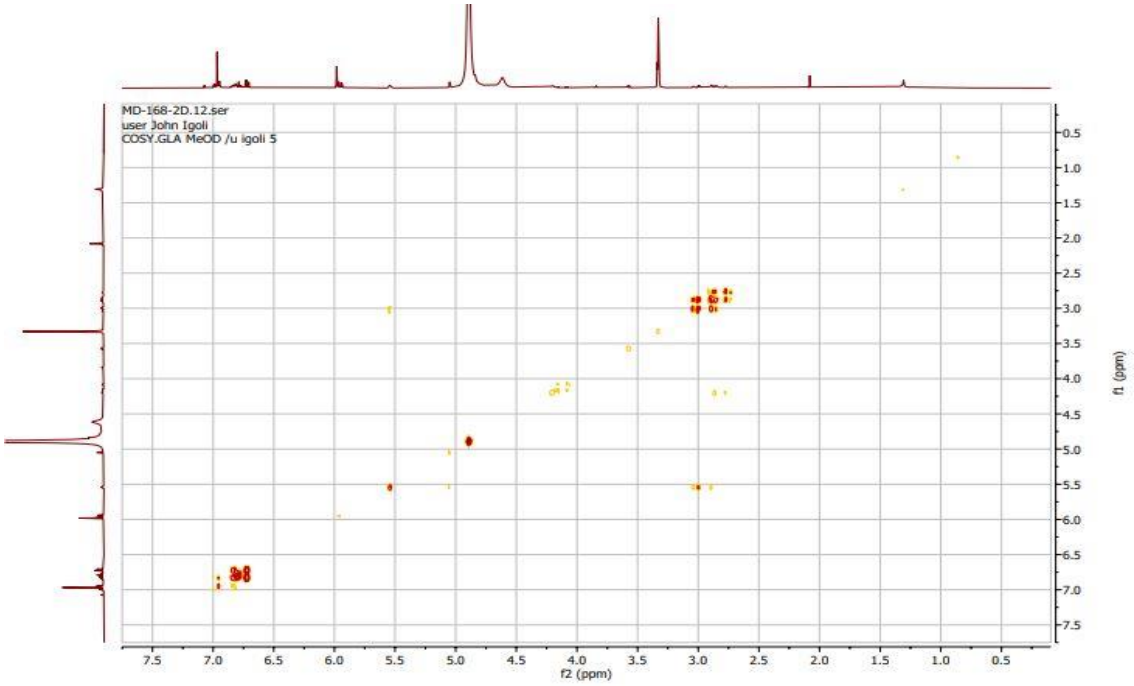
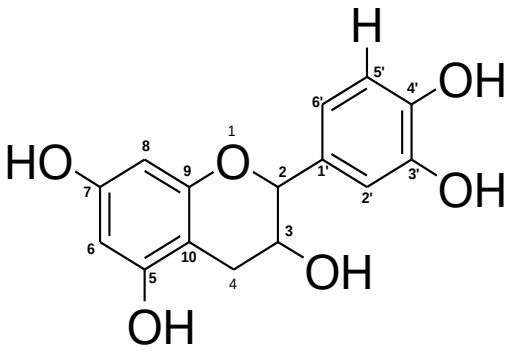


Figure 6: COSY spectrum MD168

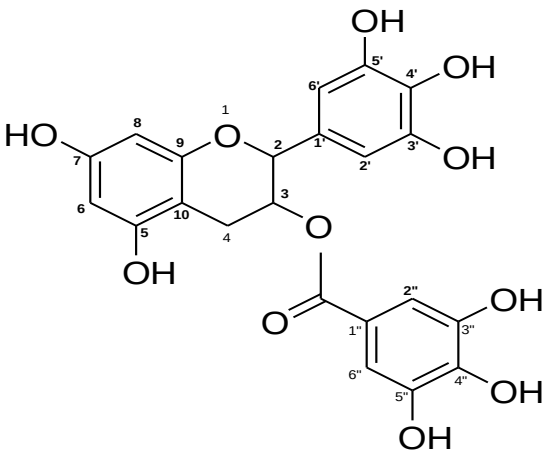
3.2 DISCUSSION

Compound MD168 which appeared reddish brown and gave a positive reaction on ferric chloride test, indicating the presence of flavonoids (Evans, 2002) was characterized using LC-MS, 1D and 2D NMR spectroscopic methods as mixture of catechin and catechin-3-0-gallate. The LC-MS Spectrum (Figure 1) showed $[M+H]^+$ m/z of 291.08 (291.1 in the literature) and $[M]^+$ m/z of 290.07 (where M is the molecular ion for the molecular formula $C_{15}H_{14}O_6$). This is in line with the result of Araya-Farias *et al.* (2014) for catechin.

	^{13}C-NMR of Catechin		**^{1}H-NMR of Catechin**		**^{13}C-NMR of Catechin-3-O-gallate**		**^{1}H-NMR of Catechin-3-O-gallate**	
	ME168	**Davis *et al.* (1996)**	**ME168**	**Davis *et al.* (1996)**	**ME168**	**Davis *et al.* (1996)**	**ME168**	**Davis *et al.* (1996)**
Position	δ_C	δ_C	δ_H	δ_H	δ_C	δ_C	δ_H	δ_H
2	78.49	82.76	4.62	4.56	77.24	78.80	5.05	5.15
3	68.60	68.38	4.08	3.99	66.60	70.46	5.54	5.40
4	27.86	28.84	α 2.99, β 2.53	4α 2.91, 4β 2.53	25.47	24.20	α 2.85, β 2.78	α 2.89, β 2.76
10	98.70	100.69		-	98.01	99.24		
6	95.18	96.19	5.99	6.02	95.03	96.47	6.00	6.07
8	94.52	95.50	5.95	5.88	NO	95.61	5.98	5.99
5	156.46	157.23			NO	157.25		
7	156.60	157.77			NO	158.08		
9	156.27	156.95			155.27	156.32		
1’	130.89	132.27			130.06	131.47		
2’	113.94	115.27	6.95	6.90	113.73	114.30	6.98	6.92
3’	144.55	145.66			NO	145.82		
4’	144.92	145.73			NO	145.89		
5’	114.63	115.74	6.83	6.80	114.52	115.99	6.82	6.79
6’	117.99	120.04	6.73	6.76	NO	119.13	6.75	6.79
1”					120.08	121.79		
2”					108.84	109.98	7.05	7.05
3”					NO	146.00		
4”					138.41	138.93		
5”					NO	109.98		
6”					108.84	146.00	7.05	7.05
CO					166.23	166.04		



Catechin



Catechin-3-0-gallate

^{1}H-NMR spectrum (400Hz, acetone-$_{d6}$) showed range of signals in pairs forming crowded peaks across the spectrum while ^{13}C-NMR spectrum showed a series of twin peaks indicating the presence of a mixture of similar compounds. In the ^{1}H-NMR spectrum, the pair of doublet of doublets between δ_H 2.7 and 3.0 were observed which are due to the protons H-4α and H-4β on carbons 4 (C-4) of the two compounds (Figure 2). Carbon-4 peaks of the two compounds are observed on the most up field position at δ_C 27.86 (for catechin) and δ_C 25.47 (for catechin-3-O-gallate) (Figure 3). This is confirmed by HSQC spectrum where these and all other protons on their respective carbons were observed (Figure 4). The catechin-3-0-gallate differ from catechin with a gallate group attached to the OH group on C-3 by removing the proton of the OH. Additional signals of the gallate group are observed: signal at δ_C 166.23 of the carbonyl group and δ_C 120.08 of the quaternary carbon at position 1" (Figure 3). These peaks are similar to those observed in Davis *et al.* (1996) for catechin and catechin-3-O-gallate (Table 1). Above information is supported by correlations observed for the two compounds on both COSY and HMBC (Figure 6 and 5) The comparison of the two compounds with the literature is given in (Table 1).

Catechin was previously isolated from the different parts of *V. paradoxa*: from MeOH and H_2O leaf extracts (Sinan *et al.*, 2020), MeOH kernel (Steven *et al.,* 2003; Zhang *et al.*, 2014; Zhang *et al.*, 2018), MeOH stem bark (Talla *et al.,* 2016; Eyong *et al.,* 2018) and from MeOH root (Nyemb *et al.*, 2018) of *V. paradoxa*. While catechin-3-O-gallate was isolated from MeOH kernel extract by Steven *et al.* (2003) and from stem and leaf methanol extracts (Sinan *et al.,* 2020). However, extensive literature review has shown that this is the first time catechin and catechin-3-O-gallate were isolated from the ethyl acetate extract of the stem bark of *V. paradoxa*.

CONCLUSION

In the current study, the stem bark of *V. paradoxa* was subjected to successive extractions using hexane, ethyl acetate and ethanol. Chromatographic studies of the ethyl acetate extract afforded a fraction which was characterized using LC-MS, ^{1}H and ^{13}C 1D NMR, proton-carbon correlations (HSQC and HMBC), proton-proton correlation (^{1}H-^{1}H COSY) experiments and by comparison with the literature as a mixture of two known types of flavanol of flavonoid class: catechin and catechin-3-O-gallate. Both compounds were previously isolated from the other parts and extracts of *V. paradoxa* and were reported to be active against strains which make them potential precursors new drug synthesis.

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List of Abbreviations

V. paradoxa: Vitellaria paradoxa
NMR: Nuclear Magnetic Resonance
1D: One Dimensional
2D: Two Dimensional
LC-MS: Liquid Chromatography- Mass Spectroscopy
HSQC: Heteronuclear Single Quantum Coherence
HMBC: Heteronuclear Multiple Bond Correlation
^{1}H-^{1}H COSY: Proton-Proton Correlation Spectroscopy
DCM: Dichloromethane
MeOH: Methanol
MeOD: Deuterated Methanol
Acetone-$_{d6}$: Deuterated Acetone
^{1}H-NMR: Proton Nuclear Magnetic Resonance
^{13}C-NMR: Carbon-13 Nuclear Magnetic Resonance
δ_H: Proton Chemical Shift
δ_C: Carbon-13 Chemical Shift
HCl: Hydrochloric Acid
m/z: Mass to Charge Ratio

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: Not applicable

Competing interests: The authors declare that they have no competing interests

Funding: Not applicable

Authors' contributions: Conceptualization, H.L.G, A.I., M.S.Y.; methodology, H.L.G., A.I., M.S.Y.; data curation, M.S.Y., A.I.; writing—original draft preparation, M.S.Y; writing—review and editing, all authors; supervision, H.L.G., A.I., U.K.J. All authors have read and agreed to the published version of the manuscript.

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