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Structure Determination of Compounds from the Stem of Equisetum Ramosissmum

Diriba Borena Hunde [a*], Dominic Ravichandran [a], Zelalem Abdisa [a], Chaltu File [b] and Desalegn Borena [c]

[a] Department of Chemistry, College of Natural and Computational Sciences, Wollega University, P.O. Box-395, Nekemte, Ethiopia.
[b] Horo Guduru Wollega Zone, Shambu Referral Hospital P. O. Box-88, Shambu, Ethiopia.
[c] Horo Guduru Wollega Zone, Shambu Preparatory School, P. O. Box-88, Shambu, Ethiopia.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

This study was carried out to isolate the major constituents of stem of *Equisetum* Ramosissmum. The dimethyl ether extract was isolated by column chromatography to give one pure compound. The compound was identified as Isophthalic acid 1-(5- methyl-hexyl) ester 3-pentyl ester. The structures of the compounds were elucidated by means of 1H-NMR, 13C-NMR and DEPT-135 spectral data and comparison with literature reports.

Keywords: Chemical constituents; some major compounds from stem of Equisetum Ramosissmum.

**Corresponding author: E-mail: diribaborena@gmail.com;*

1. INTRODUCTION

"Plants are important in human's life and fulfill his every day needs. Different plant parts like root, stem, flower, leaves, fruit, twigs and modified plant organs are used as food, Shelter, clothing, transportation, fertilizer, flavors, fragrances, ornamental, medicine, cosmetic, etc. throughout the ages of humans. According to some observation some animals like chimpanzees utilize a number of plant species for medicinal use" [1].

"The discovery of plants that serve as medicine (medicinal plant) in different parts of the world is important to agriculture and medicine sectors, in establishment of new directions towards propagation of alternative medicinal plant that offer better economic and social benefits. Historically, natural products have been used since ancient times and in folklore for the treatment of many diseases and illnesses the ancient civilizations from different countries such as Chinese, S. Africa, Thailand, Borneo, Cameroon, Uganda and Madagascar" [2].

Today, these medicines still represent an important part of healthcare in some countries to cover the basic health needs in the developing countries like Africa. "In many Africans countries traditional medicines from medicinal plants are sold in marketplace or prescribed by traditional healer. For example, mandrake was prescribed for pain relief, turmeric possesses blood clotting properties, roots of the endive plant were used for treatment of gall bladder disorders, and raw garlic was prescribed for circulatory disorders" [3].

"The world health organization (WHO) reported that 80% of the world's populations depend on traditional medicine and a major part of traditional therapies involve the use of medicinal plant extracts or their active constituents" [3].

"Medicinal plants used traditionally throughout the world to treat many illnesses like malaria, diabetes, respiratory and urinary tract infections, cough, fever, diarrhea, abdominal pains, pneumonia, conjunctivitis, oral and tooth wounds etc. and also used for birth control and psychic problems and often exhibit a wide range of biological and pharmacological activities, such as anti-bacterial and anti-fungal properties. Due to the need for development of new compounds with better pharmacological activities, dependence on medicinal plants grew increasingly as scientists continuously exploited them for isolation of bioactive compounds" [4]. Medicinal plants belonging to the genus *Equisetum* are very often used in traditional medicine for tea and other therapeutic products. They are highly efficient in treating urinary and respiratory tract infection, cardiovascular diseases, medical skin conditions etc. Many Costa Rican species, *Equisetum myriochaetum*, is used medicinally as a treatment for human kidney trouble, as a diuretic, as a styptic especially in urinary trouble, and in the fomenting of septic inflammations. The sap from this plant is reportedly used to relieve toothache and is also applied to the wound after extraction [5].

On this regard *Equisetum Ramosissmum* is a medicinal plant which is popularly called "horsetail", and locally called "Riga bofa" is used by traditional medicine practioners as wound treatment, toothache, treating urinary tract infection, cardiovascular diseases, respiratory tract infection and medical skin conditions [6].

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Identification

The Stems of *Equisetum ramosissimum* Local name *(Riga bofa)* in Afaan Oromo was collected from Amuru village, Horro Guduru Zone, Oromia Region, which is 383 km west of Addis Ababa. The plant was identified by Prof. Legesse Negash and specimen was deposited at the National Herbarium (Voucher Diriba Borena 002/2015) the Department of Biology, Addis Ababa University.

2.2 Experimental Procedures Extraction

90 g (divided into three parts) of powdered stem of *Equisetum ramosissimum* were extracted by using dimethyl ether, chloroform, acetone and methanol (350 ml for each) in Hot Continuous Extraction (Soxhlet) apparatus.

2.3 Isolation of Compounds

According to the TLC analysis methanol: ethyl acetate was 8:2. Depending on this ratio of solvent selected column chromatography was packed with petroleum ether after absorbing the dimethyl ether extract with silica gel and concentrated on rotary evaporator. The extract was applied on column and eluted with

increasing polarity of petroleum ether/etoac solvent mixtures. Elution of the column by pe: EOAC is given in Fig. 1.

2.4 Spectroscopic Analysis

Pure fractions from column chromatography were characterized by using IR, UV and nuclear magnetic resonance (1H-NMR 13C-NMR, DEPT and the spectra were recorded in CDCl3 and DMSO-d6 with Tetramethylsilane (TMS) as internal standard for calibrating chemical shift. Complete structure determination was achieved by comparing the IR and NMR data obtained with that in literature.

2.4.1 Isolation of compounds form PE-EtOAC extract of stem of Equisetum Ramosissmum

Compound PE-2 (11 mg) was isolated using CC from non-polar fraction as blue black solid. TLC analysis by PE: EtOAC (6:4) showed a single spot with $R_{f\ =}$ 0.7 staining red under UV light. This compound is most likely one of a non-polar and ^{1}H NMR, ^{13}C NMR and DEPT-135 spectral data of the compound is given in Table 1.

The ^{1}H NMR spectrum (Appendix A and, Table 1) of the compound showed peaks δ 0.902 (6H, and δ integrating for two methyl protons. A methylene signals appeared at δ 1.277 and δ 1.608 each integrating for two protons each. Other methylene signals appeared at δ 1.019 and 2.046 each integrating for two protons each. Ox methylene signals appeared at δ 4.189. Aliphatic methine proton peaks appeared at δ 1.831. Furthermore, the two overlapped signal appeared at assigned for δ 7.748 the two olefinic methane protons. One signal is observed δ7.763 for olefinic methine proton.

The ^{13}C NMR and DEPT-135 (Appendix B, C and Table 1) indicated that compound PE-2 has 20 carbon atoms. The spectra showed two aliphatic methyl carbons at δ 22.71 and one aliphatic methyl carbon at δ 14.14. Two aliphatic methylene carbons at δ 23.75 and 29.71, two methylene carbons at 31.94, two methylene carbon at δ 30.36., two other methylene carbon at δ 66.20. Two ox methylene carbon atoms appeared at δ 68.19 one aliphatic methine at δ 28.83, two olefinic methine at δ 128.83., one methine at δ 130.94, and one two olefinic quaternary carbon at δ 132.37, two ester carbonyl carbons at δ 167.00.

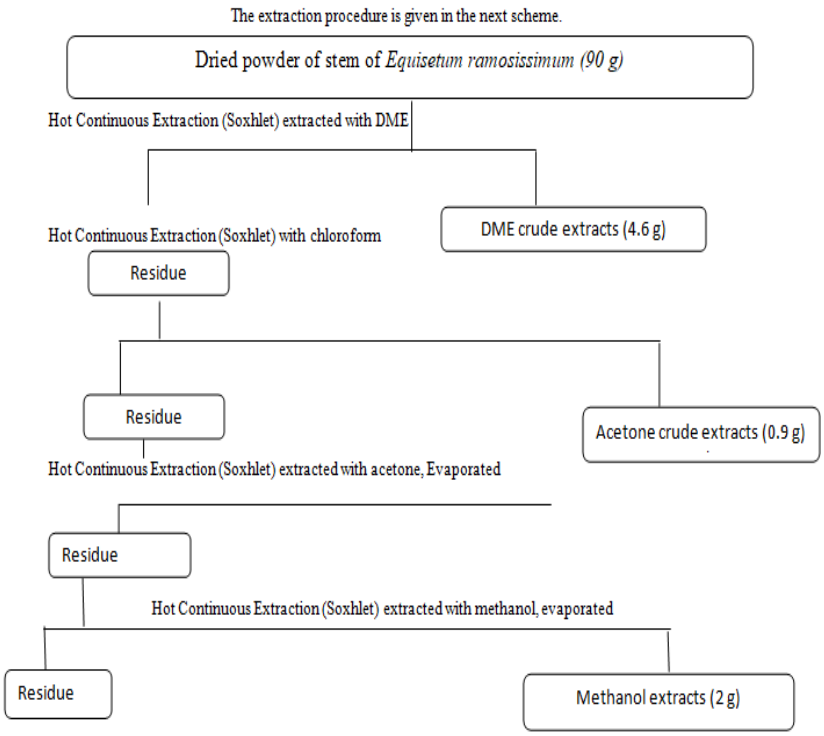
Based on the above NMR data the next structure was proposed for the compound PE-2.

3. RESULTS AND DISCUSSION

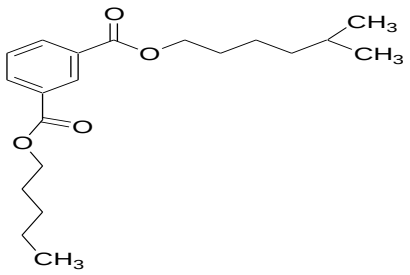
The dried powder of stem of *equisetum Ramosissmum* was extracted with dme, chloroform, acetone and methanol successively. The dme extract was isolated by column chromatography to give one pure compound. The chemical structure was characterized on the basis of NMR spectral data, including ^{1}h-nmr and ^{13}c-nmr in comparison with literature values. The compound pe-2 (11 mg) was identified as Isophthalic acid 1-(5- methyl-hexyl) ester 3-pentyl ester.

Table 1. ^{1}H NMR, 13C NMR and DEPT-135 for PE-2

No	^{1}H δ (ppm)	^{13}C δ (ppm)	DEPT-135 δ (ppm)	Remark
1		167.00		COO-
2		132.37		
3	7.763	130.94	130.95	CH=
4	7.748	128.82	128.82	CH=
5	4.189	68.19	68.20	CH_2-O
6	2.046	31.94	31.94	CH_2
7	1.019	30.36	30.36	CH_2
8	1.608	29.71	29.71	CH_2
9	1.831	28.83	28.93	CH
10		27.73	27.73	
11	1.277	23.75	23.75	CH_2
12	1.002	22.71	22.71	CH_3-CH
13		19.39	19.39	
14	0.902	14.14	14.16	CH_3



Scheme 1. Hot continuous extraction (soxhlet) procedure



Isophthalic acid 1-(5-methyl-hexyl) ester 3-pentyl ester

Fig. 1. Tentative structure of compound PE-2

To improve the yield of medium polarity crude extract (acetone) and high polarity crude extract (methanol), the availability of solvent for the extraction should be maximize to isolate more phytochemicals from the plant.

4. CONCLUSION

Therefore, it would be better if further study is carried on identifying high extracting solvent, to elucidate the complete and correct structure of pure compounds additional experimental data is recommended and further study is carried on identifying phytochemical used as cleaning tooth, treating toothache and wounds after tooth extraction and toxicity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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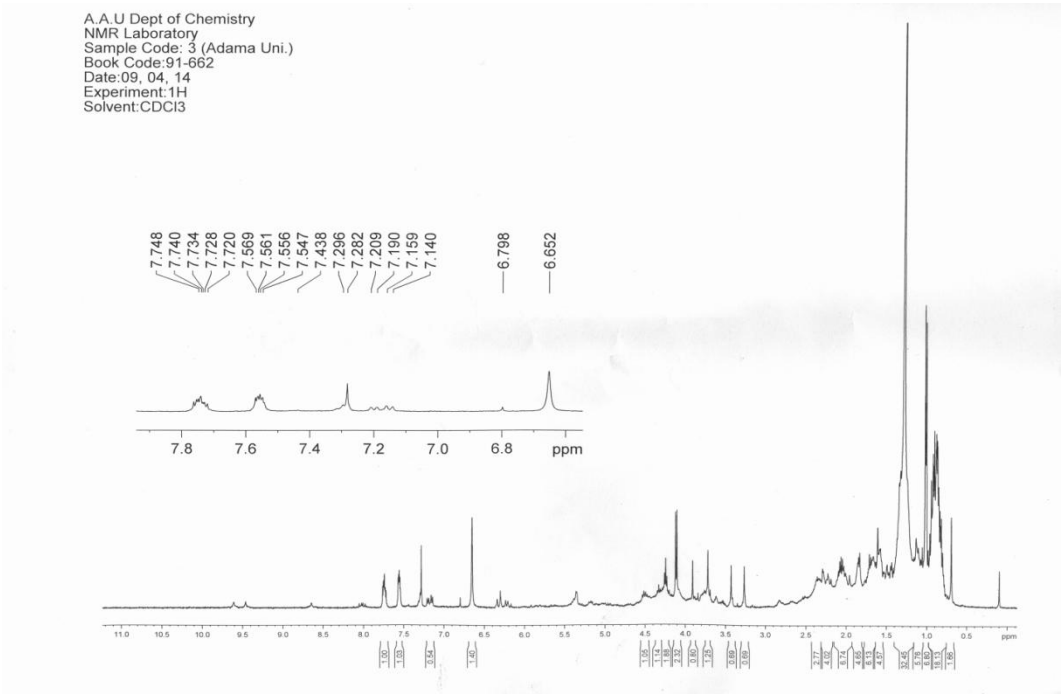
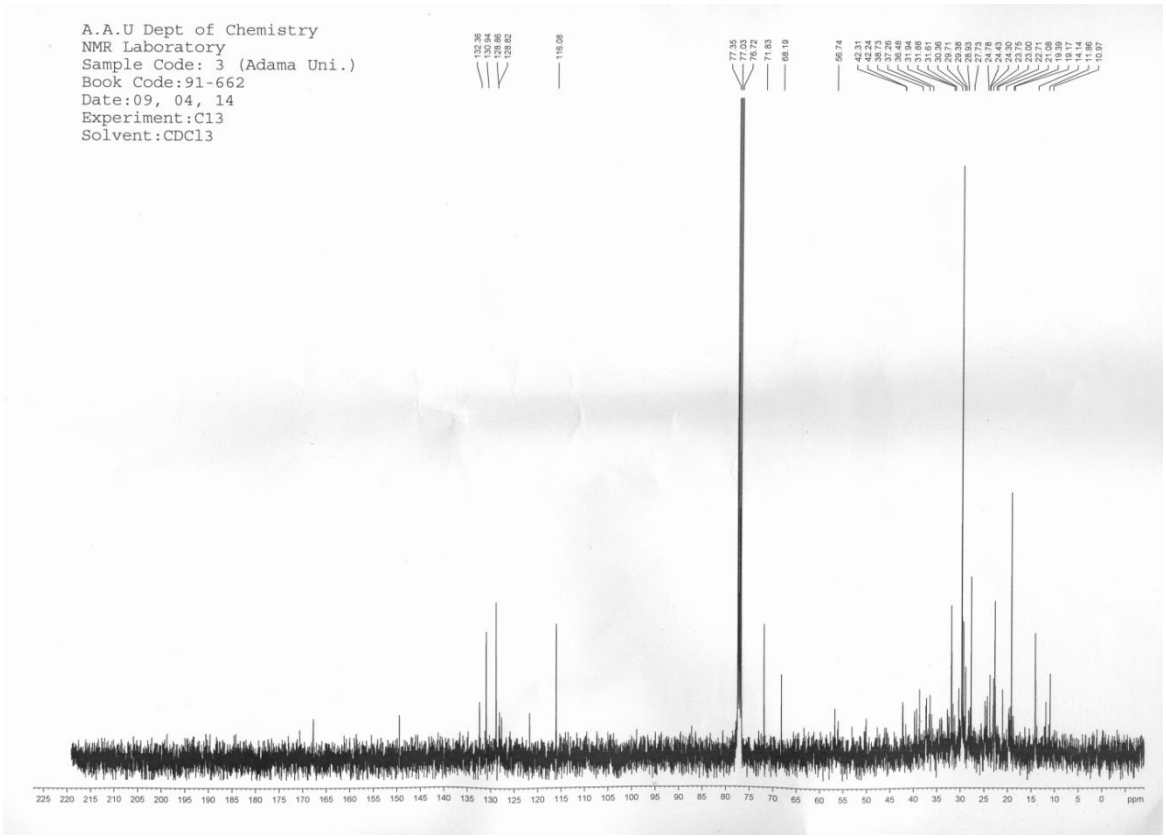
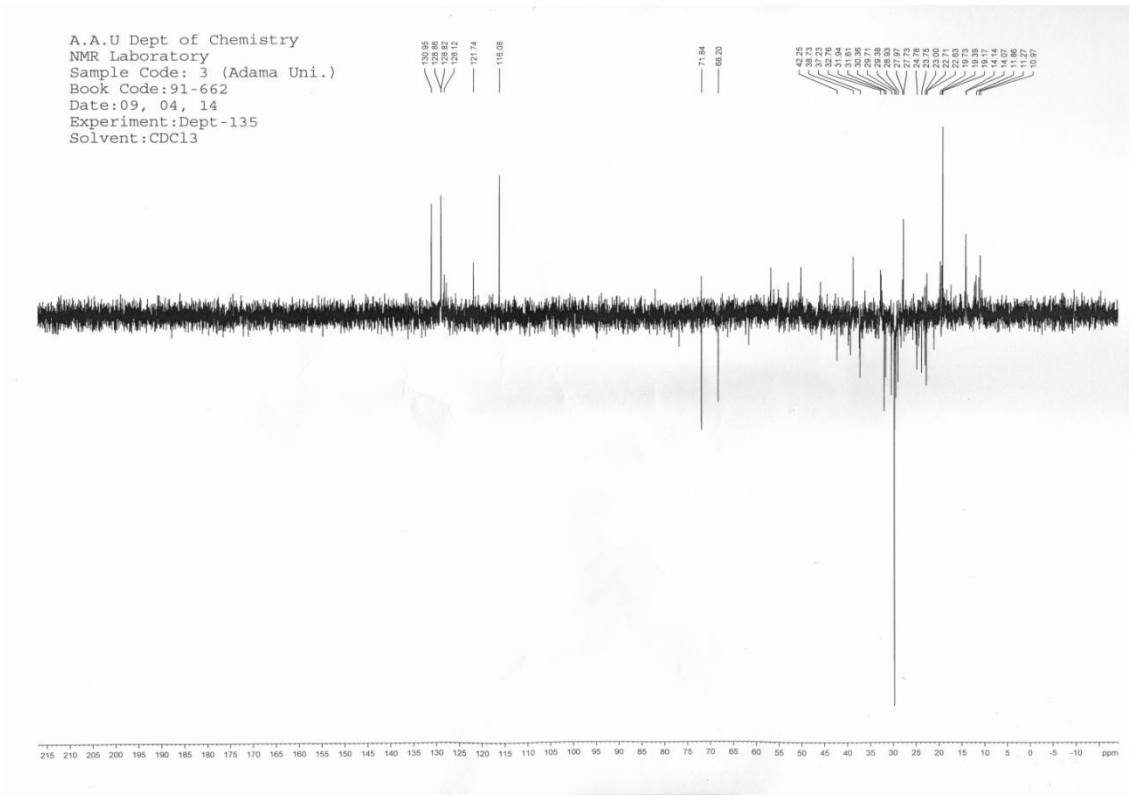
APPENDIX



Appendix A. ^{1}H NMR Spectrum of compound PE-2 in $CDCl_3$



Appendix B. ^{13}C NMR Spectrum of compound PE-2 in $CDCl_3$



Appendix C. DEPT-135 Spectrum of compound PE-2

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