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DETERMINATION OF NEW CHEMICAL CONSTITUENTS OF SAADA (CYPERUS ROTUNDUS L) RHIZOMES

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ABSTRACT

To further purify the compounds the extract was absorbed on Polyamide gel. Bioactivity guided fractionation of *C. rotundus* led to isolation and characterization of six compounds by These compounds were isolated by using different chromatographic techniques and their structures were identified by(TLC method GCMS, UV, IR and ^{1}D NMR spectrum ($C^{13}$and ^{1}H-NMR) Moreover, GCMS analysis four known compounds(Stigmasterol(8.36%),4-Isopropyl-1,6-dimethyl-decahydronaphthalene (4,34%), 2H-Cyclpropanaphthalene-2-one(9.10%),1H-Cyclopropa{e} azulene (2.98%)) and two known compounds NMR analysis(3-isobutyl-Octahydro-naphthalene Tetradecahyl- pentaphenanthrenoate,).

INTRODUCTION

The essential oil was fractionated into fractions rich in hydrocarbons and oxygenated compounds using silica gel column chromatography (CC). These hydrocarbons were further separated through CC and TLC (thin-layer chromatography), resulting in isorotundene, (−)-cyperaa-2,4(15)-diene, norrotundene, cyperadione, and α-patchoulane-type sesquiterpene (Sonwa and König, 2001, Elezabeth and Arumugam 2014 Hikino,. and Aota 1998, Hsu, 1926, **Jeong**, Inagaki,. and Higuchi2000).[1-4] The main class of compounds including sesquiterpenes, epoxides, ketones, monoterpenes, aliphatic alcohols, and aromatic compounds are identified in *C. rotundus* essential oils

(Sonwa and König, 2001).[6] Gas chromatography (GC) and combined GC/MS (mass spectrometry) analysis identified 32 different components in the oil, forming about 98.6% of the total composition (Kilani., et al 2007).[5]

MATERIALS AND METHODS

Successive Extraction of the Roots

Weighed 100g of the rhizomes were successively extracted with petroleum ether, chloroform, ethyl acetate and methanol using Soxhlet Apparatus. Extraction was carried out for about 4 hours for petroleum ether, 12 hours for chloroform, and 8 hours for Ethyl acetate and 2 hours for methanol. Extract were then dishes and left under for to dryness. The yield percentage of each extract was calculated as follow.

Weight of extract X100/ weight of the crude powder plant.

Column Chromatography (CC)

Glass column of 90 cm length and 4 cm internal. Diameter and silica gel were used. Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan.

Packing of the Column

A small piece of cotton was situated at the end of the column with glass rod. Column was filled with 450 g of silica and clouted with n hexane until the silica macerated. Air bubbles were removed by lapping the outer surface of column with rubber rod.

NMR spectroscopy

^{1}H NMR spectroscopy

The NMR spectrum of isolated products was obtained on a DRX-400 (1H 400 MHz) instrument from BRUKER (Karlsruhe, Germany). The internal standard was tetra methyl silane Proton chemical shifts are reported in ppm (d) relative to internal Tetra methyl saline (TMS, d 0.0 ppm), or with the solvent reference relative to TMS employed as an internal standard ($CDCl_3$, d 7.26 ppm). Data are reported as follows: chemical shift (multiplicity [singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m)], coupling constants [Hz], integration).

C^{13}NMR spectroscopy

Carbon NMR spectra were recorded on a Varian 400 (100 MHz) or 500 (125 MHz) spectrometers with complete proton decoupling. In the Spectrum Devices Laboratory at King

Abdulaziz University in Jeddah, Saudi Arabia. Carbon chemical shifts are reported in ppm (d) relative to TMS with the respective solvent resonance as the internal standard (CDCl3, d 77.0 ppm). All NMR spectra were acquired at ambient temperature.

FT-IR spectroscopy

The infrared (IR) spectrum of the 5and 6 extract was measured (as KBr discs) in the range of 400–4000 cm−1 on FT-IR spectrophotometer (Shimadzu FTIR-8400 S, Kyoto, Japan). In the Spectrum Devices Laboratory at King Khalid University in abha, Saudi Arabia. The important IR bands, such as υ (C–N), υ (O–H), υ (CH), υ (C = C), υ (NH), υ (CO) and (CH) symmetric and asymmetric stretching, and stretching frequencies were studied to determine the presence of functional groups in the 5 and 6 extracts.

Polyamide Column

Column was prepared by macerated 90 g of polyamide power in about 25 ml distilled water for one hour then was poured in the column and kept till complete homogeneous. Extract after mixed with silica gel was poured at the top of the column and eluted with (water, methanol, ethyl acetate and chloroform). The graduation of solvent was 20% in each time. fraction was collected in 25 ml test tube and monitored with thin layer chromatography on silica gel using n-butanol: acetic acid: water (12 :3 :5) as solvent system. The plates were detected under UV lamp and sprayed with vanillin sulpuric acid plates containing the similar spots were combined together and allowed to dry then weight.

RESULTS AND DISCUSSION

Fraction of Column Chromatography

Fraction of column chromatography of the *C.rotundus* Powdered extracted from rhizomes by using different solvents as (methanol, ethyl acetate, chloroform and petroleum ether).

(methanol: MeOH, ethyl acetate: E.A, chloroform: $CHCL_3$ and petroleum ether: P.E, Fr. No: Fraction number and Wt.: weight).Column chromatography is method used to purify individual chemical compounds. It is often used for preparative applications on scales from micrograms u to kilograms. the fraction done by solvent eluent using $CHCL_3$ and MeOH showed good weight and good percentage more than that which used other solvent eluent as E.A. Solvent eluent mixture MeOH and E.A mixture gave high yield and percentage (2.566, 23.32%) and (2.369, 21.54%) respectively in fraction number (183-188) and (189-195). Fractions which having high weights were Fraction 71-119 (12,944 g) and Fraction 13 (2,566

g) and 14(2,369) while the Fractions which have least weight are Fraction 25 (0.051 g) and fraction 26 (0.001 g).

Table (1): Polyamide column fraction.

fraction	Weight	Limit	Elution
1	0.04g	1-80	Water
2	0.032g	81-100	Water- Methanol
3	0.172g	101-106	Methanol- ethyl acetate
4	0. 62g	107-126	Chloroform
5	0. 45g	127-138	Chloroform-P.Ether

Table (1) five main extractions were purified by method involving polyamide column chromatography. The extraction/separation technique was validated using TLC method. Solvent eluent mixture MeOH and E.A mixture gave high yield (0.172g), CHCL3 (0. 62g) and -P.E (0. 45g) respectively in fraction number (101-106), (107-126) and (127-138). These weights were suitable for the rest of the analysis techniques. ol {9:1}

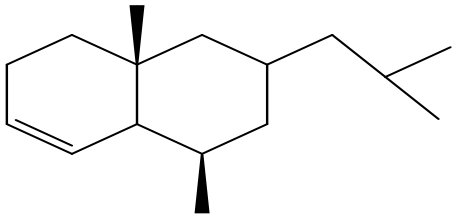
Structure Elucidation of compound MH-5

The structure of the compound is given below:

IR ν_{max} (KBr): The FT-IR spectra exhibited bands 2995, 2924, 1729, 1717, 1360, 1241, 1149, 500, and 402cm−1 from which the presence of alkanes, alkenes, hydroxyl and carboxyl groups was inferred. The IR spectrum of the compound included a diagnostic peak at 2995 cm−1. Was assigned to aromatic C–H stretching. The peak appearing at 2924 cm−1 was assigned to CH3– and –CH2– stretching (alkanes). The peak appearing at 1717cm−1 was assigned to C = C stretching (alkene). The peak appearing at 1241 cm−1 was assigned to ether. The peak appearing at 1149cm−1 was assigned to CH3. The peak appearing at 500 cm−1 was assigned to alkene and the peak appearing at 402 cm−1 was assigned to alkyl halide. Structure compound MH-5 was elucidated by ^{13}CNMRand ^{1}HNMR. Spectra showed the presence of four terminal methyl's at δ, 1.25, 1.41, 1.33 and 2, 09 are assigned for H_{11}', H_{12}, H_{15}, and H_{16}", respectively. two signals at δ 6.59, and 6.60 and due to olefinic protons are assigned for H3'and H4', respectively ^{1}H-NMR data also showed five multiplets signals attributed to methylene protons at δ1.28, 1.42, 2.00,2.15, and 2.95 are assigned toH_1, H_2, H_7, H_9 and H_{13} respectively. Four methine signals at δ 3.64, 4.29, 1.29 and 0.88 are assigned to H_5, H_6, H_8 and H_{14} respectively.

^{13}CNMR data revealed clear two carbons contributed in the double bonds at δ123.53 and 124.13 are assigned to C_3 and C_4 respectively. ^{13}CNMR data showed intense signals

attributed to four methine signals at δ 33.70, 29.67, 30.20 and 14.14(olefenic carbon), are assigned to C_5, C_6, C_8 and C_{14}respectively. Five methylenes signals at δ28.98, 31.45, 23.19, 29.38 and 115.91 are assigned to C_1, C_2, C_7, C_9 and C_{13} respectively. Four methyl groups at δ26.71, 22.71 31.63 and 31.94 assigned to C_{11}, C_{12}, C_{15} and C_{16} respectively. Data obtained from ^{1}H and ^{13}C spectrum showed that this compound has molecular formula of $C_{16}H_{28}$ corresponding to MW equal to 220 On the bases of these evidences the structure of MH-5 has been established as $C_{16}H_{28}$



3-Isobutyl-1,4a-dimethyl-1,2,3,4,4a,5,6,8a-octahy

Fig (3): 3-isobutyl-Octahydro- naphthale.

Structure Elucidation of compound MH-6

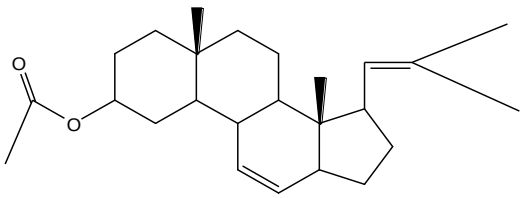
The structure of the compound is given below:

IR ν_{max} (KBr): The FT-IR spectra exhibited bands 3250,3000, 2950, 2858, 1729, 1486, 1366, 1273, 1241, 1149,755 and 500cm^{-1} from which the presence of alkanes, alkenes, hydroxyl and carboxyl groups was inferred. The IR spectrum of the compound) included a diagnostic peak at 3250 cm^{-1} hydroxyl group. 3000 $cm^{-1}$was assigned to aromatic C–H stretching. The peak appearing at 2950 cm^{-1} was assigned to CH_3– and –CH_2– stretching (alkanes). The peak appearing at 2858 cm^{-1} was assigned to OH (alcohol) symmetric stretching. The peak appearing at 1729cm^{-1} was assigned to C = C stretching (alkene). The peak appearing at 1486 cm^{-1} was assigned to COO^{-1} stretching (fatty acid ester). The peak appearing at 1366 cm^{-1} was assigned to C–O group. The peak appearing at 1241 cm^{-1} was assigned to ether. The peak appearing at 1149cm^{-1} was assigned to CH_3. The peak appearing at 755 cm^{-1} was assigned to alkene and the peak appearing at 500 cm^{-1} was assigned to alkyl halide.

Compound MH-6 was identified by NMR. The^{1}H and ^{13}C NMR spectral. The ^{1}HNMRspectrum showed proton appeared as triplet of and three olefinic protons appeared at δ 5.06 (H_{11}, m), and δ 4.71 (H_{20}, m) five methyl groups resonating at δ 1.25, 1.28, 1.57, 2.09, and 2.13 ppm are assigned H_{18}, H_{19}, H_{22}, H_{23}, and H_{25} respectively. ^{1}H-NMR data also showed multiplets signals attributed to methlene protons at δ2,00, 2.58, 2.61, 2,88, 4,20 ,7,52,

and 7.69 are assigned H_1, H_2, H_4, H_8, H_9, H_{14}, and H_{15} respectively. Spectra showed the presence of five terminal methine at δ0.83, 0.91, 1.41, 1.42 and 2.04 are assigned H5, H_6, H_7, H_{13}, and H_{17} respectively. one signals at oxygenated methylene region at δ 3.00 are assigned H_3.

^{13}CNMR data showed intense signals attributed to two quaternary carbons including ester carbonyl group at δ 165.3 is assigned to C_{24} two carbons contributed in the double bonds at δ129.75 and 130.93are assigned to C_{11} and C_{12} respectively. ^{13}CNMR data showed intense signals attributed to seven methane signals at δ 76.87(attached to oxygen), δ 38.73, 37.11, 31.94,31.63, 23.75 and 124,09 (olefenic carbon), are assigned to C_3, C_5, C_6, C_7, C_{13} C_{16} and C_{20} respectively. Seven methylene's signals at δ22.71, 23.00, 28.93, 29.38, 30.20, 30.71, and 115.91), are assigned to C_1, C_2, C_4, C_8, C_9, C_{14} and C_{15} respectively. Five methyl groups at δ 13.74, 14.14, 19.20, 19.20, and 14.07, assigned to C_{18}, C_{19}, C_{22}, C_{23}, and C_{25} respectively On the bases of these evidences the structure of MH-6- has been established as with molecular formula $C_{25}H_{37}O_2$, the identification of the MH-6- was confirmed by comparing its chromatographic and spectral data (TLC and NMR) with reference sample.



Acetic acid 5,14-dimethyl-15-(2-methyl-propenyl)-2,3,4,5,6,7,8,9,10,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-2-yl ester

Fig (6): Tetradecahyl-penta phenanthrenoate.

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REFERENCE

1. Elezabeth V.D. and Arumugam S. (2014) GC-MS Analysis of ethanol extract of Cyperus rotundus leaves. International Journal of Current Biotechnology, 2(1): 19-23.
2. Hikino, H. and Aota, K. (1998).Sesquiterpenoids. Part 52. 4A, 5A-oxidoeudesm-1 I-En3A-O1, sesquiterpenoid of Cyperus rotundus. Phylochemistry, 15: 1265-1266.
3. Hsu, H.Y. (1926). The Chemical Constituents of Oriental Herbs. Oriental Healing Arts Institute. Long Beach, CA: 932.

4. Jeong, S.J., Miyamoto, T., Inagaki, M., Kim, Y.C. and Higuchi, R. (2000). Rotundines AC, three novel sesquiterpene alkaloids from Cyperus rotundus. J. Nat. Prod., 63(5): 673-675.
5. Kilani S, Ledauphin J, Bouhlel I, Ben Sghaier M, Boubaker J, Skandrani I, Mosrati R, Ghedira K and Barillier D, L (2007). Comparative study of Cyperus rotundus essential oil by a modified GC/MS analysis method. Evaluation of its antioxidant, cytotoxic, and apoptotic effects Unité de Pharmacognosie/Biologie Moléculaire 99.
6. Sonwa, M.M and Konig, W.A. (2001).Chemical study of the essential oil of Cyperus rotundus. Phytochemistry, 58(5): 799-810.