Original Research Article

A STUDY OF PHYTOCHEMICAL CONSTITUENTS IN *CARALLUMA QUADRANGULA*

Abstract:-

Four known secondary metabolites, namely Glochidonol (1), 3β,14β-dihydroxy-14β-pregn-5-en-20-one (2), 3β-Hydroxystigmast-5-en-7-one (3), & Stigmasterol (4). The structures of these compounds were established by usual spectroscopic methods. This is the first report of 3β-Hydroxystigmast-5-en-7-one from *Caralluma quadrangula* stem.

Keyword:- *Caralluma quadrangula , Glochidonol,* 3β,14β-dihydroxy-14β-pregn-5-en-20-one , 3β-Hydroxystigmast-5-en-7-one *& Stigmasterol.*

Introduction:

For thousands of years, medicinal plants have played an important role throughout the world in treating and preventing a variety of diseases. The medically significant genus *Caralluma* is widely studied for its stem and fruits. It belongs to the family Asclepiadaceae, which comprises 200 genera and 2500 species (1). The genus *Caralluma* comprises about 200 species distributed throughout Africa and Asia. The majority of these species are indigenous to the Indian sub-continent and Arabian Peninsula (2). Certain species of *Caralluma* are edible and form part of the traditional medicine system of many countries (3). These are commonly used in folk medicine as remedies to treat wide variety of diseases and health conditions (4). *C. arabica* is traditionally used as an emollient and diuretic in United Arab of Emirates. It is also used to treat liver diseases, diabetes and hypertension. The flowers of *C. arabica* are applied externally for wounds and cuts, while the juice of the stem is given to sick people to speed convalescence of burns, itchy skin and sunburns (5,6). In the Indian state Andhra Pradesh, *C. attenuata* (Wight) is eaten raw as an antidiabetic agent, while the juice of the plant along with black pepper is recommended in the treatment of migraine (7). The diverse applications of *Caralluma* plants in folk medicine have prompted the phytochemical and biological investigations of their constituents (8). The key phytochemical ingredients in *Caralluma* are pregnane glycosides, flavone glycosides, megastigmane glycosides, bitter principles, triterpenes and saponins (9,10,11,12).

Materials and Methods:-

A.. General experimental procedures:-

All chemicals were purified by following regular procedures [13,14] and all chemicals used Analytical Reagent grade.

B.. Plant material:-

Stems a of *Caralluma quadrangula* (Asclepiadaceae) were collected from Sana'a 2014. The plant identified by Dr. Hessen Ibrahim. A voucher specimen of plant was deposited in Herbarium, Department of Phytochemistry.

C.. Extraction and Isolation:-

Shade dried stems were powdered and sieved. Afterwards the powder was stored in air tight container. The powder was weighed and extracted with soxhlet extractor by using solvents of polarity from non-polar end to polar end (Hexane, Chloroform, and Methanol) with successive solvent extraction. To concentrate the extracts and removal of final traces of solvent, rotary evaporate was used [15,16]. Then, recrystallization

was done to purify the crude extracts. Melting point was taken by using Fisher-John apparatus. The ^{1}H NMR and ^{13}C NMR spectra were taken on Bruker 100 MHz and 400 MHz, spectrometer, using an internal standard like TMS. Mass spectra were recorded by using ZAB-HS mass spectrometer.

C..1. Test for alcohol:-

Small amount of crude extract was dissolved in 0.5 ml of dioxane. The obtained solution was added to 0.5 ml of ceric ammonium nitrate reagent. Then about one ml of dioxane was added and shaken. Yellow to red color formation indicates the existence of an alcoholic hydroxyl group [17].

C..2. Libermann-Burchard test:-

Few drops of acetic anhydride were added to the extract and boiled. Concentrated sulphuric acid was added to the above cooled solution. Presence of sterols is confirmed from formation of a brown ring at the junction of two layers and green color in upper layer [17].

C..3. General extraction and isolation:-

The air-dried powder (1100g) of stems of C. quadrangula was extracted (Soxhlet) with solvents (3X, 8 hours each) and the combined extracts evaporated to give a brown gummy residue (6 g). This extract was subjected to silica gel flash column chromatography (FCC) with chloroform containing increasing percentages of methanol as eluent and each collected fraction was 20 ml. Fractions 1-5 were combined and rechromatographed by C.C. to yielded JJ1 (5.0 mg) identified as Glochidonol, (1), (hexane-EtOAc), JJ2 (7.0 mg) identified as 3β,14β-dihydroxy-14β-pregn-5-en-20-one (2), JJ3 (7.8 mg) identified as 3β-Hydroxystigmast-5-en-7-one (3) & JJ5 (6.0 mg) identified as Stigmasterol (4). All the isolated compounds were identified by comparison with data from previous NMR and mass spectra.

Glochidonol (1). White powder (5.0 mg), mp 227-230 ^{0}C. ^{1}H NMR (CDCl3, 300 MHz): δ 4.71, 4,60 (2H, s, H-29a, b), 3.26 (1H, dd, J= 4.76, 11.00 Hz), 0.63, 0.65, 0.80, 0.90, 0.94, 1.00, 1.10 (each 3H, s, Me×7). ^{13}C NMR (CDCl3, 100 MHz): δ 214.67 (C-3), 150.1 (C-20), 105.5 (C-29), 80.1 (C-1), 55.5 (C-5), 50.6 (C-9), 48.5 (C-18), 48.1 (C-19), 43.2 (C-17), 43.0 (C-14), 41.0 (C-8), 40.2 (C-22), 39.0 (C-13), 38.9(C-4), 37.3 (C-10), 35.8 (C-16), 34.5 (C-7), 30.0 (C-21), 28.2 (C-23), 27.6 (C-15), 27.5 (C-12), 25.3 (C-2), 21.1 (C-11), 19.5 (C-30), 18.5 (C-6), 18.2 (C-28), 16.3 (C-25), 16.2 (C-26), 15.6 (C-24), 14.7 (C-27).

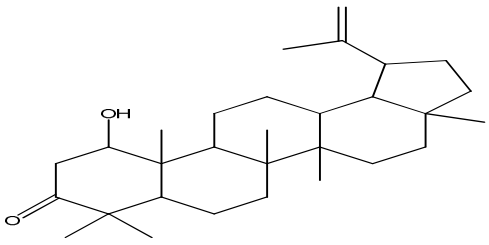

Fig.1. Glochidonol

3β,14β-dihydroxy-14β-pregn-5-en-20-one (2). Mp: 200 -201 0C. ^{1}H-NMR (300 MHz, CDCl3): δ 1.05 (3H s), 1.07 (3H s), 1.31 (3H, s), 1.45 (2H m), 1.50 (2H, m), 1.54 (1H, m), 1.60 (1H m), 1.75, 1.8 (2H), 1.98 (2H, dd J1 =12.5; J2 = 5.5 Hz), 2.28 (1H,q, J = 7.8Hz), 2.43 (2H, m), 3.7 (H, m, H-3), 4.12 (H, s, H-14) 5.16 (1H, s, H- 6). ^{13}C-NMR (75 MHz, CDCl3): δ 36.6 (C-1), 28.0 (C-2), 75.0 (C-3), 36.6 (C-4), 143.1 (C-5), 121.9 (C-6), 36.1 (C-7), 53.2 (C-8), 50.4 (C-9), 36.5 (C-10), 25.3 (C-11), 25.2 (C-12), 43.7 s, C-13), 78.1 (s, C-14), 32.8 (C-15), 25.4 (C-16), 53.6 (s, C-17), 15.5 (C-18), 15.3 (C-19), 121.5 (s, C-20), 30.1 (C-21).

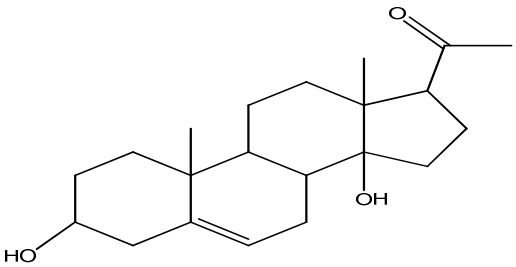

Fig.2. 3β,14β-dihydroxy-14β-pregn-5-en-20-one

3β-Hydroxystigmast-5-en-7-one (3). Colorless granules from CHCl3/MeOH; mp: 128 ^{0}C. EIMS m/z (rel. int.): 428 [M]+ (100), 410 (20), 395 (32), 287 (18), 247 (10), 192 (30). ^{1}H-NMR (300 MHz, CDCl3): δ 0.67 (3H, s, H-18), 1.17 (3H, s, H-19), 3.67 (1H, m, H-3α), 5.69 (1H, d, J = 1.8 Hz, H-6). ^{13}C-NMR (75 MHz, CDCl3): δ 36.28 (t, C-1), 31.16 (t, C-2), 70.52 (d, C-3), 41.78 (t, C-4), 166.10 (s, C-5), 124.23 (d, C-6), 202.85 (s, C-7), 45.56 (d, C-8), 49.93 (d, C-9), 38.36 (s, C-10), 21.20 (t, C-11), 38.81 (t, C-12), 43.08 (s, C-13), 49.93 (d, C-14), 26.54 (t, C-15), 28.11 (t, C-16), 54.48 (d, C-17), 11.94 (q, C- 18), 17.30 (q, C-19), 36.07 (d, C-20), 18.91 (q, C-21), 33.93 (t, C-22), 26.35 (t, C-23), 45.87 (d, C-24), 29.69 (d, C-25), 18.76 (q, C-26), 19.79 (q, C-27), 23.03 (t, C-28), 11.94 (q, C-29).

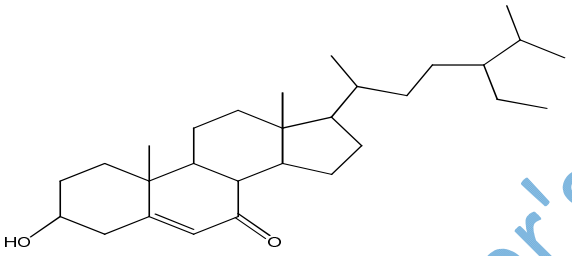

Fig.3 3β-Hydroxystigmast-5-en-7-one

Stigmasterol (4). ^{1}H NMR (100 MHz, CDCl3): δ 0.82 (10H, q, J=7.19 Hz, Me-21γ, Me-26, Me-27, Me-29), 0.93 (1H, t, J=5.40 Hz Me-21β), 5.35 (1H, d, J=4.62 Hz, H-22), 1.02 (8H, d, J=6.57 Hz , H-1α, 1β, Me-18, Me-19β, Me-19γ, Me-21α), 1.18 (6H, m, J=6.95 Hz 11β, H-12 β, H-12β, H-14, H-15 β, Me-19 β, H-28 β, H-28 β,), 5.15 (1H, q, J=7.86 Hz, H-23), 1.46 (8H, m, J=5.91 Hz H-2 β, H-2 β, H-8, H-9, H-11α, H-15 β, H-16 β, H-16 β), 1.67 (3H, d, J=10.35 Hz H-15α, H-17, H-25), 1.85 (2H, d, J=10.05 Hz, H7α, 7β), 2.03 (3H, m, J=8.07 Hz, 3-OH, H-20, H-24), 2.26 (2H, t, J=8.13 Hz, H-4α, 4β), 3.52 (1H, m, J=5.09 Hz H-3), 5.01 (1H, q, J=7.85 Hz H-6). ^{13}C NMR (CDCl3): δ 12.20 (C-29), 12.33 (C-21), 19.10 (C-27), 20.10 (C-26), 21.25 (C-19), 21.40 (C-18), 21.60 (C-11), 24.48 (C-16), 25.53 (C-15), 29.04 (C-28), 31.74 (C-8), 32.01 (C-7), 32.01 (C-25), 32.01 (C-12), 36.62 (C-1), 37.38 (C-20), 39.8 (C-2), 40.62 (C-10), 42.32 (C-13), 42.39 (C-9), 50.27 (C-4), 51.36 (C-14), 56.06 (C-24), 56.98 (C-17), 71.88 (C-3), 121.8 (C-6), 129.38 (C-22), 138.44 (C-23), 140.86 (C-5).

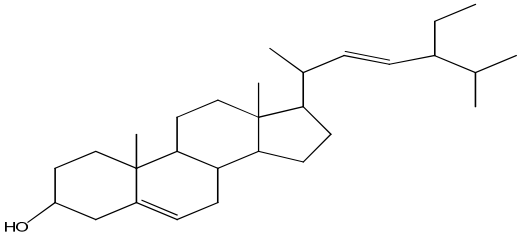

Fig.4. Stigmasterol

Result and Discussion:-

Compound (1). is white powder. The ^{1}H NMR spectrum showed seven tertiary methyl singlet's and one secondary hydroxyl group. It also showed olefinic protons at δ 4.71 and 4.60. ^{13}C NMR of the compound showed 30 signals for the terpenoid of lupine skeleton which was represented by seven methyl groups. The carbon bonded to the hydroxyl group C-1 appeared at δ 80.1, while the alkenic carbons appeared at δ 149.1 and 105.5.

Compound (2). Was isolated as white needle-like crystals, mp 200-201^{0}C. The mass spectral data of the compound gave a molecular formula C_{21} $H_{32}O$, m/z 412 M^{+}. ^{1}H-NMR showed signals for three angular methyl singlet's at δ 1.05, 1.07 and 1.31.

The proton of H-3 appeared as a multiplet at δ 3.53 and singlet at δ 4.12. It also showed olefinic protons at δ 5.16. ^{13}C NMR showed twenty one carbon signal including three methyles, nine methylenes, five methins and five quaternary carbons. The alkenes carbons appeared at δ 143.1 and 121.9. The significant signal for the 3β,14β-dihydroxy-14β-pregn-5-en-20-one would be the signals for two carbon attached to hydroxyl group, which is C-3 and C-14 that appeared at δ75.0 & 78.1.

Compound (3). was isolated as Colorless granules. The mass spectral data of the compound gave a molecular weight m/z 428. ^{1}H NMR spectra showed the presence of tow methyl's appeared at δ 0.67 & 1.17.The proton of H-3 appeared as amultiplet at δ 3.67. It also showed olefinic protons at δ 5.69. ^{13}C NMR showed twenty one carbon signal including three methyles, ten methylenes, nine methins and four quaternary carbons. The alkenes carbons appeared at δ 166.10 & 124.23.

Compound (4). ^{1}H NMR spectrum showed peaks primarily in the up field region. However, two signals corresponding to olefinic region were observed with high chemical shifts values. A multiplet at δ 3.52 is characteristic to a carbinylic proton of sterol moiety. Peaks in low absorption field i.e., at δ 5.35 , 5.15 & 5.01 correspond to two and one ethylene protons respectively present on C22, C23 and C6. High intensity Peaks at δ 1.02 and 0.82 are corresponding to methyl groups (Me-19, Me-18, Me-26, Me-27 and Me-29). ^{13}C NMR spectrum shows the presence of 6 methyl, 9 methylene, 11 methine and 3 quaternary carbons. Signals at δ 140.86 and 121.8 correspond to double bond. Attachment of β-hydroxyl group to C3 is visible from a peak at δ 71.88 [31]. High intensity peak at δ 21.21 represent angular methyl carbons – C19 and C18. γ-Gauche interaction enhances the screening of C-18 leading to lower chemical shift.

Conclusion :-

The isolation and identification Glochidonol, *3β,14β-dihydroxy-14β-pregn-5-en-20-one, 3β-Hydroxystigmast-5-en-7-one* & Stigmasterol *from the stems of Caralluma quadrangula*. The work was carried out by means of various physical (solvent extraction, column chromatography, radial chromatography, preparative TLC and malting points) and spectral techniques

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