



RESEARCH ARTICLE

A STUDY OF PHYTOCHEMICAL CONSTITUENTS IN *CARALLUMA QUADRANGULA*

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Article Info:



Article History:

Received: 4 February 2020

Reviewed: 9 March 2020

Accepted: 27 April 2020

Published: 15 May 2020

Cite this article:

Al-Mahweety JAN, Azzam SHS, Alyahawi A. A study of phytochemical constituents in *Caralluma quadrangula*. Universal Journal of Pharmaceutical Research 2020; 5(2):28-31. <https://doi.org/10.22270/ujpr.v5i2.385>

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Abstract

Objectives: *Caralluma* belongs to the family Asclepiadaceae, native to the Indian sub-continent have different medicinal properties and used in folk medicine as remedies to treat wide variety of diseases and health conditions.

Objective of present study was to isolate, analyze and identify the phytochemicals composition from stem of *Caralluma quadrangula*.

Methods: Extractions of the chemical components was carried out by different chromatographic techniques. The phytochemical characterizations were evaluated by nuclear magnetic resonance and mass spectrometry.

Results: The quantitative phytochemical analysis of this species exhibited the presence of three compounds, *Glochidonol* (5 gm), Dihydroxy-14-pregn-5-en-20-one (9.8 gm), Hydroxystigmast-5-en-7-one (7.8 gm) and Stigmasterol (5.7 mg). Hydroxystigmast-5-en-7-one, report from *Caralluma quadrangula* stem as first time.

Conclusion: The present study is strengthen for the discovery three pure chemical compounds from *C. quadrangula*.

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

INTRODUCTION

Thousands of years, medicinal plants have an important role during the world in treating and preventing a diversity of diseases. *Caralluma* is belongs to the family Asclepiadaceae, medically important genus widely studied for its stem and fruits. *Caralluma* classification to 200 genera and 2500 species¹. *Caralluma* genus have about 200 species spread in Africa and Asia. The common of these species are native to the Indian sub-continent and Arabian peninsula². Most species of *Caralluma* are edible and form part of the traditional medicine system in the word³. Usually used in folk medicine as remedies to treat wide variety of diseases and health conditions⁴. Also used to treat liver diseases, diabetes and hypertension. The *Caralluma* flowers are useful superficially 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 migraine⁷.

The diverse applications of *Caralluma* plants in folk medicine have prompted the phytochemical and biological investigations of their constituents⁸. The key phytochemical ingredients in *Caralluma* are pregnane glycosides, flavone glycosides, megastigmane glycosides, bitter principles, triterpenes, and saponins⁹⁻¹².

MATERIALS AND METHODS

General experimental procedures:-

All active constituent were purified by following standard procedures^{13,14} and all chemicals used Analytical Reagent grade.

Plant material:-

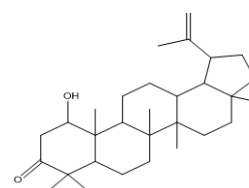


Figure 1: *Glochidonol*.

Stems a of *Caralluma quadrangula* (Asclepiadaceae) from Sana'a were collected (2014). The plant identified

by Dr. Hessen Ibrahim. Deposited the sample of plant in Herbarium, Department of Phytochemistry.

Extraction and Isolation:-

Shade dried stems were powdered. The powder was stored in air close container. The powder was weighed and extracted with soxhlet extractor by using solvents (Hexane, Chloroform, and ethanol). To concentrate the

extracts and removal of solvent, rotary evaporate was used^{15,16}. Then, purify the crude extracts. By using Fisher-John apparatus melting point was taken. The ¹H NMR and ¹³C NMR spectra done by Bruker 100 MHz and 300 MHz, spectrometer, using an internal standard like TMS. Mass spectra were recorded by using ZAB-HS mass spectrometer.

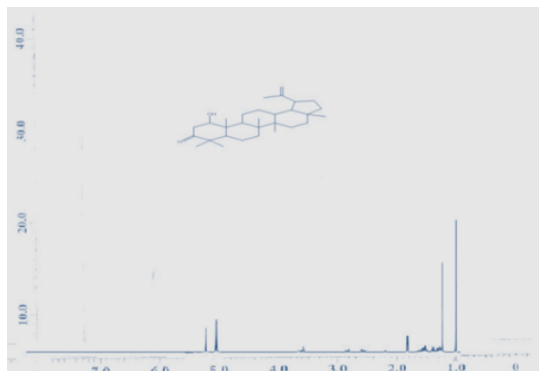


Figure 2: ¹H NMR of Glochidonol (1).

Test for alcohol

Dissolved little amount of crude extract was in 0.5 ml of dioxane. The solution was added to 0.5 ml of ceric ammonium nitrate reagent. Then one ml of dioxane was added and stunned. Yellow to red color formation gives an alcoholic hydroxyl group¹⁷.

Libermann-Burchard test

Acetic anhydride drops were added to the extract and boiled. Concentrated sulphuric acid was added to the above cooled solution. Presence of sterols gives a brown ring at joint of two layers and green color in upper layer¹⁷.

General extraction and isolation

The air-dried powder (1100 g) of stems of *Caralluma quadrangula* was Soxhlet with solvents (3X, 8 hours each) and evaporated the combined extracts to give a dark brown residue (6 g) subjected the extract to silica gel flash column chromatography (FCC) with chloroform containing increasing percentages of ethanol as eluent. Fractions 1-5 were combined and purification by C.C. to yield JJ1 (5.0 mg) known as Glochidonol, (1), (hexane-EtOAc), JJ2 (9.8 mg) known as 3,14-dihydroxy-14-pregn-5-en-20-one (2), JJ3 (7.78 mg) known as 3-Hydroxystigmast-5-en-7-one (3) and JJ5 (5.7 mg) known as Stigmasterol (4). All the isolated compounds were recognized by relationship with spectroscopy data.

Glochidonol (1): White powder (4.9 mg), mp 226-230 °C. ¹H NMR (CDCl₃, 300 MHz): δ 4.71, 4.60 (2H, s, H-29a, b), 3.26 (1H, dd, J = 4.76, 11.00 Hz), 0.61, 0.63, 0.81, 0.92, 0.93, 1.01, 1.10 (each 3H, s, Me×7). ¹³C NMR (CDCl₃, 100 MHz): δ 214.68 (C-3), 149.08 (C-20), 105.46 (C-29), 80.09 (C-1), 55.51 (C-5), 50.62 (C-9), 48.54 (C-18), 48.10 (C-19), 43.12 (C-17), 43.02 (C-14), 41.08 (C-8), 40.24 (C-22), 39.10 (C-13), 38.29 (C-4), 37.30 (C-10), 35.85 (C-16), 34.53 (C-7), 30.01 (C-21), 28.25 (C-23), 27.63 (C-15), 27.54 (C-12), 25.27 (C-2), 21.06 (C-11), 19.52 (C-30), 18.48 (C-6), 18.21 (C-28), 16.26 (C-25), 16.20 (C-26), 15.61 (C-24), 14.66 (C-27).

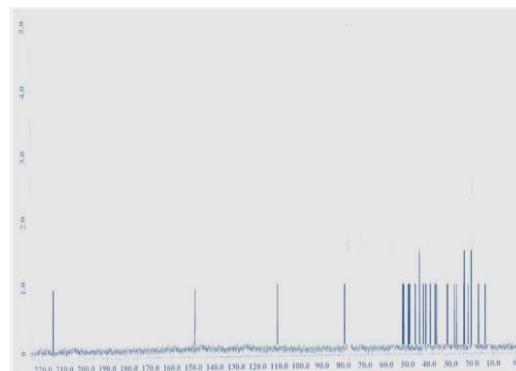


Figure 3: ¹³C NMR of Glochidonol (1).

3,14-dihydroxy-14-pregn-5-en-20-one (2): Mp: 200 - 201 °C. ¹H-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, 1.80 (2H), 1.97 (2H, dd J1 = 12.5; J2 = 5.5 Hz), 2.27 (1H, q, J = 7.8 Hz), 2.42 (2H, m), 3.54 (H, m, H-3), 4.12 (H, s, H-14) 5.17 (1H, s, H-6). ¹³C-NMR (75 MHz, CDCl₃): δ 36.64 (C-1), 28.01 (C-2), 75.16 (C-3), 36.62 (C-4), 143.06 (C-5), 121.86 (C-6), 36.10 (C-7), 53.19 (C-8), 50.41 (C-9), 36.48 (C-10), 25.34 (C-11), 25.21 (C-12), 43.65 s, C-13), 78.30 (s, C-14), 32.81 (C-15), 25.43 (C-16), 53.6 (s, C-17), 15.45 (C-18), 15.32 (C-19), 121.47 (s, C-20), 30.07 (C-21).

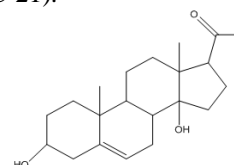


Figure 4: 3β,14β-dihydroxy-14β-pregn-5-en-20-one.

3-Hydroxystigmast-5-en-7-one (3): Colorless granules; mp: 127.5 °C. EIMS m/z (rel. int.): 428 [M]⁺ (100), 410 (19), 395 (31), 287 (17), 247 (09), 192 (31). ¹H-NMR (300 MHz, CDCl₃): δ 0.66 (3H, s, H-18), 1.15 (3H, s, H-19), 3.68 (1H, m, H-3α), 5.67 (1H, d, J = 1.8 Hz, H-6). ¹³C-NMR (150 MHz, CDCl₃): δ 36.27 (C-1), 31.15 (C-2), 70.51 (C-3), 41.77 (C-4), 166.12 (C-5), 124.20 (C-6), 202.83 (C-7), 45.55 (C-8), 49.92 (C-9), 38.32 (C-10), 21.21 (C-11), 38.82 (C-12), 43.08 (C-13), 49.93 (C-14), 26.54 (C-15), 28.11 (C-16), 54.48 (C-17), 11.94 (C-18), 17.30 (C-19), 36.07 (C-20), 18.91 (C-21), 33.93 (C-22), 26.34 (C-23), 45.87 (C-24), 29.68 (C-25), 18.75 (C-26), 19.78 (C-27), 23.02 (C-28), 11.93 (C-29).

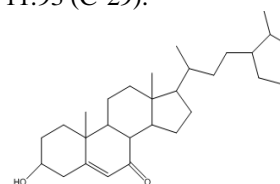


Figure 5: 3β-Hydroxystigmast-5-en-7-one

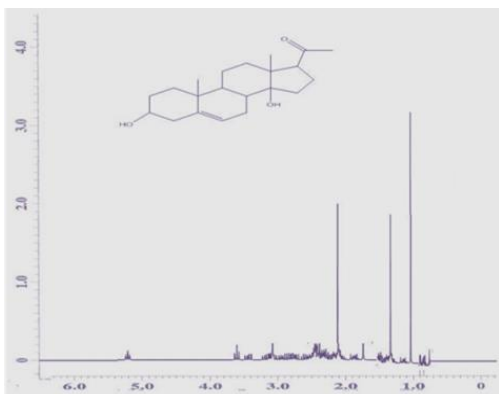


Figure 6: ^1H NMR of dihydroxy-14 β -pregn-5-en-20-one (2).

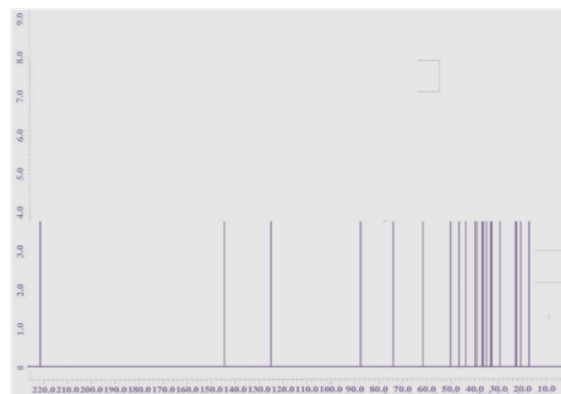


Figure 7: ^{13}C NMR OF dihydroxy-14 β -pregn-5-en-20-one (2).

Stigmasterol (4): ^1H NMR (100 MHz, CDCl_3): δ 0.85 (10H, q, $J=7.19$ Hz, Me-26, Me-27, Me-29), 0.91 (1H, t, $J=5.40$ Hz Me-21), 5.34 (1H, d, $J=4.62$ Hz, H-22), 1.09 (8H, d, $J=6.57$ Hz , H-1, Me-18, Me-19, Me-21), 1.18 (6H, m, $J=6.95$ Hz, H-12, H-14, H-15, Me-19, H-28), 5.10 (1H, q, $J=7.86$ Hz, H-23), 1.48 (8H, m, $J=5.91$ Hz, H-2, H-8, H-9, H-11, H-15, H-16), 1.65 (3H, d, $J=10.35$ Hz, H-17, H-25), 1.80 (2H, d, $J=10.05$ Hz, H7), 2.12 (3H, m, $J=8.07$ Hz, 3-OH, H-20, H-24), 2.29 (2H, t, $J=8.13$ Hz, H-4), 3.51 (1H, m, $J=5.09$ Hz, H-3), 4.97 (1H, q, $J=7.85$ Hz, H-6).

^{13}C NMR (CDCl_3): δ 12.21 (C-29), 12.32 (C-21), 19.09 (C-27), 20.07 (C-26), 21.30 (C-19), 21.35 (C-18), 21.55 (C-11), 24.46 (C-16), 25.52 (C-15), 29.03 (C-28), 31.74 (C-8), 32.00 (C-7), 32.01 (C-25), 32.04

(C-12), 36.61 (C-1), 37.36 (C-20), 39.83 (C-2), 40.61 (C-10), 42.32 (C-13), 42.38 (C-9), 50.26 (C-4), 51.30 (C-14), 56.04 (C-24), 56.97 (C-17), 71.81 (C-3), 121.82 (C-6), 129.36 (C-22), 138.40 (C-23), 140.81 (C-5).

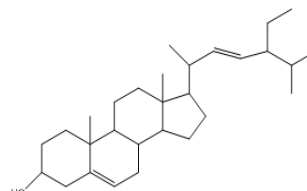


Figure 8: Stigmasterol.

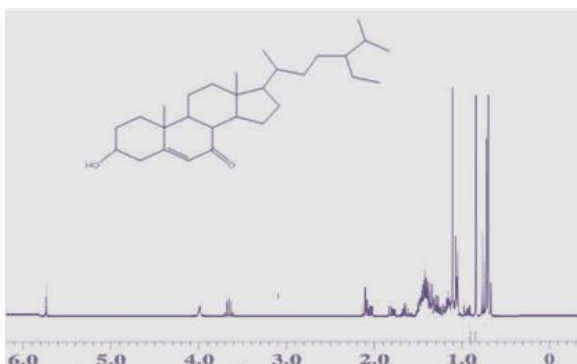


Figure 9: ^1H NMR of Hydroxystigmast-5-en-7-one (3).

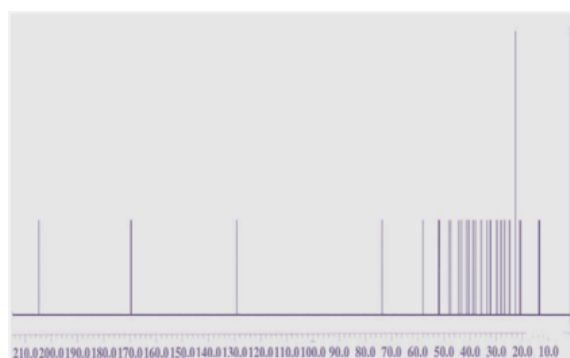


Figure 10: ^{13}C NMR of Hydroxystigmast-5-en-7-one (3).

RESULTS AND DISCUSSION

Compound 1: White powder, ^1H NMR scale showed seven tertiary methyl groups and one secondary hydroxyl group. Olefinic protons appeared at δ 4.71 and 4.60. ^{13}C NMR of the Glochidonol showed hydroxyl group C-1 appeared at δ 80.09, 30 signals for the terpenoid of lupine skeleton which have seven methyl groups. The carbon double bonded of alkenic carbons appeared at δ 149.08 and 105.46.

Compound 2: Was white needle-like crystals, MP 200-201 $^\circ\text{C}$, molecular formula $\text{C}_{21}\text{H}_{32}\text{O}$, m/z 412 M^+ . ^1H -NMR for three methyl singlet's at δ 1.05, 1.07 and 1.31. H-3 proton appeared as a multiple at δ 3.53 and singlet at δ 4.12. Also have olefinic protons at δ 5.17. The ^{13}C NMR showed alkenes carbons appeared at δ 143.06 and 121.86 and have twenty one carbon signal

with three methyles, nine methylenes, five methins and five quaternary carbons. Carbon bond to hydroxyl group is C-3 and C-14 that appeared at δ 75.2 and 78.3.

Compound 3: Were colorless granules, MS data of the compound gives m/z 428. ^1H NMR spectrum showed tow methyl groups appeared at δ 0.66 and 1.15. The H-3 proton appeared at δ 3.68 as a multiplet and showed olefinic protons at δ 5.67. ^{13}C NMR showed twenty one carbon signals with three methyles, ten methylenes, nine methins and four quaternary carbons. Double bond carbons appeared at δ 166.12 and 124.20.

Compound 4: ^1H NMR data showed c proton of sterol part at δ 3.51 as multiple. Signals at δ 5.34, 5.10 and 4.97 correspond to two and one ethylene protons correspondingly present on C22, C23 and C6. Also have Peaks at δ 1.09 are corresponding to methyl groups (Me-18, Me-19, Me-21) and 0.85 are

consequent to methyl groups (Me-26, Me-27 and Me-29). ^{13}C NMR spectrum have six methyl, nine methylene, eleven methine and three quaternary

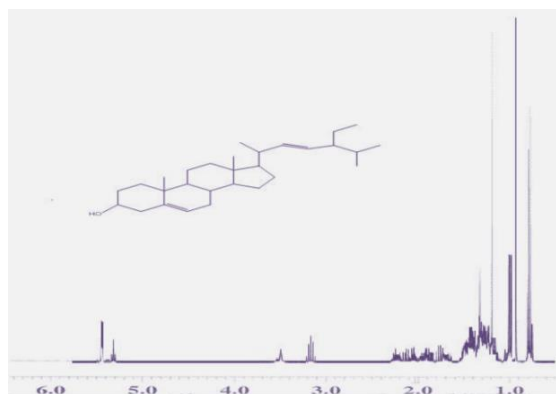


Figure 11: ^1H NMR of Stigmasterol (4).

carbons. Double bond Signals appeared at δ 140.81 and 121.82. Proton additional of β -hydroxyl group to C3 showed a peak at δ 71.81.

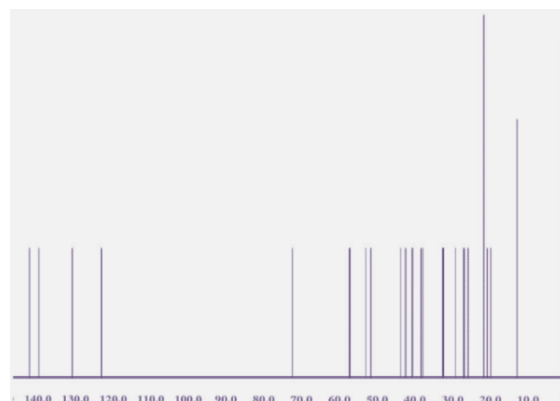


Figure 12: ^{13}C NMR of Stigmasterol (4).

CONCLUSIONS

The isolation and identification Glochidonol, 3,14-dihydroxy-14-pregn-5-en-20-one, 3-Hydroxystigmasterol and Stigmasterol from the stems of *C. quadrangula*. The isolation, purification and analysis carried out by means of various physical (solvent extraction, column chromatography, radial chromatography, preparative TLC and melting points) and spectral techniques.

ACKNOWLEDGEMENTS

The authors extend their thanks and appreciation to the Sana'a University, Yemen to provide necessary facilities for this work.

AUTHOR'S CONTRIBUTION

Al-Mahweety JAN: writing original draft, methodology. **Azzam SHS:** investigation, formal analysis. **Alyahawi A:** conceptualization, editing. The final manuscript was read and approved by all authors.

DATA AVAILABILITY

The data supporting the findings of this study are not currently available in a public repository but can be made available upon request to the corresponding author.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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