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RESEARCH ARTICLE

PHYTOCHEMICAL PURIFICATION OF ACTIVE CONSTITUENTS ISOLATED FROM ROOT OF THE MEDICINAL HERB, *CARALLUMA QUADRANGULA*

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Abstract

Objective: Present study aim for the purification of quantitative phytochemical compounds from roots of *Caralluma quadrangula* belongs to the family Asclepiadaceae. This type of plants can be use as folk medicine to take care of wide diversity of health and diseases situation.
Methods: Preliminary phytochemical analysis for different type of chemical compounds by using various 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 four pure compounds, hydroxyoplopan-4-one (4.5 mg), dihydroxyeudesm-4(15)-ene (5.0 mg), and quercetin- rhamnopyranosyl-D-glucopyranose (Rutin) (7.0 mg).
Conclusion: From this study, it can be concluded that the species found four pure compounds from *C. quadrangula.*
Keywords: *Caralluma quadrangula*, hydroxyoplopan-4-one, dihydroxyeudesm-4(15)-ene, quercetin- rhamnopyranosyl- D-glucopyranose (Rutin).

INTRODUCTION

Medically significant genus *Caralluma* is widely studied for its stem and fruits. It is belong to the family Asclepiadaceae, which comprises 200 genera and 2500 species[1]. About 200 species belong to genus *Caralluma* distributed throughout Africa and Asia. The greater part of species are native in Indian sub-continent and Arabian Peninsula[2]. A number of *Caralluma* species use as anti-hyperglycemic goings-on of their crude extracts or their corresponding fractions[3,4]. The investigation of the chemical and biological members of genus *Caralluma*[3,5] the anti-hyperglycemic activity of the extracts, fractions of the major pregnane glycoside of the aerial parts of *C. quadrangula* was investigated in Kingdom Saudi Arabia as novel. We use the extract of *C. quadrangula* as herbal medicine in Saudi, for the treatment of freckles, diabetes, vitiligo and melasma and for thirst, hunger[5,6]. Several countries the species of *Caralluma* are fit to be eaten and variety division for the traditional medicine organization[7]. These plants can be use as folk medicine as remedies to health situation and treat large multiplicity of diseases[8].

The species of *C. arabica* use as traditionally for an emollient and diuretic In United Arab of Emirates. Also used to care for diabetes, hypertension and liver diseases. *The C. Arabica flower* used for wounds and cuts, while the juice of the stem is given to sick people to speed convalescence of burns, itchy skin and sunburns[9,10]. The *C. attenuate* species in Indian (Andhra Pradesh) use for eaten raw as an anti-diabetic agent, although the juice of the plant beside the black pepper is suggested in the treatment of migraine[11]. The different applications of *Caralluma* plants in folk medicine have prompted the phytochemical and biological investigations of their constituents[12]. The pregnane glycosides, flavone glycosides, megastigm-ane glycosides, bitter principles, triterpenes and saponins isolated from *Caralluma*[13,14,15,16].

MATERIALS AND METHODS

Purified every one of chemical constituent by subsequent standard procedures[17,18] and all chemicals used systematic Reagent evaluation.

Plant material:-

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

Extraction and Isolation:-

Shade dried roots were crushed and sieved. Next powder was stored in air closing container. Than weighed and extracted with soxhlet extractor by using solvents Chloroform with consecutive solvent extraction. To concentrate the extracts and removal of final traces of solvent than vapor[19,20]. After that, 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.

Extraction and isolation

Extracted by using Soxhlet (2 Kg) of *C. quadrangula* roots powder with solvents (3X, 8 hours each) and then evaporated collective extracts to give a brown gummy residue (8 g) after than separation and purified by silica gel flash column chromatography (FCC) with $CHCl_3$ containing increasing percentages of MeOH as eluent and collected 20 ml for each fraction. Fractions 3-10 were combined and rechromatographed by C.C. with CHCl3-MeOH (8:2) to afford JA1 (4.5 mg) identified as 10α- hydroxyoplopan-4-one (1), CHCl3-MeOH (7:3) to afford JA3 (5.0 mg) identified as 1β, 6α-dihydroxyeudesm-4(15)-ene (2), CHCl3-MeOH (6:4) to afford JA4 (5.0 mg) identified as and CHCl3-MeOH (3:7) to afford JA4 (7.0 mg) identified as quercetin- rhamnopyranosyl- D-glucopyranose (Rutin) (4). NMR data used to identified for each pure compounds.

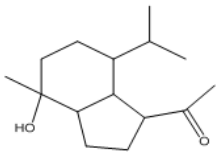

Figure 1: 10α-Hydroxyoplopan-4-one (1).

^{1}H-NMR (100 MHz, $CDCl_3$) δ: 2.75 (1H, m, H-3), 2.30 (3H, s, H-15), 1.50 (3H, s, H-13), 1.10 (3H, d, H-11), 0.87 (3H, d, H-12) ; ^{13}CNMR (MHz, $CDCl_3$) δ: 209.4 (C-14), 73.0 (C-8), 56.0 (C-3), 54.6 (C-9), 48.2 (C-5), 45.8 (C-4), 41.0 (C-7), 28.6 (C-10), 27.5 (C-1), 24.4 (C-2), 21.9 (C-6), 21.0 (C-11), 19.2 (C-13), 18.5 (C-15), 15.0 (C-12).

Dihydroxyeudesm-4(15)-ene (2). ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 5.10 (1H, brs, H-15), 5.01 (1H, brs, H-15), 3.79 (1H, t, H-6β), 3.42 (1H, dd, H-1α), 2.33 (1H, ddd, H-3α), 2.24 (1H, sept, H-11), 2.07 (1H, ddd, H-3β), 1.91 (1H, s, H-8), 1.85 (1H, ddd, H-2α), 1.75 (1H, brd, H-5α), 1.53 (1H, m, H-2β), 1.53 (1H, m, H-8), 1.43 (1H, brs, 1-OH), 1.28 (1H, m, H-7α), 1.20 (1H, m, H-9a), 1.18 (1H, m, H-9b), 1.02 (3H, d, H-13), 0.87 (3H, d, H-12), 0.72 (3H, s, H-14) ; ^{13}C-NMR (MHz, $CDCl_3$) δ: 147.4 (C-4), 108.2 (C-15), 79.1 (C-1), 67.8 (C-6), 56.4 (C-5), 50.1 (C-7), 42.1 (C-10), 36.9 (C-9), 36.1 (C-3), 32.2 (C-2), 26.5 (C-11), 21.8 (C-13), 19.1 (C-8), 16.6 (C-12), 12.0 (C-14).

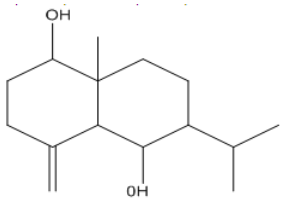

Figure 2: dihydroxyeudesm-4(15)-ene (2).

Quercetin-L-rhamnopyranosyl-(1→6)-Dglucopyranose (3). ^{1}H NMR (100 MHz, $CDCl_3$): δ 6.22 (1H, *d*, H-6), 6.40 (1H, *d*, H-8), 7.68 (1H, *s*, *H-2'*), 6.90 (1H, *d*, H-5'), 7.59 (1H, *d*, H-6'), 5.10 (1H, d, H_{glc}-1), 3.49 (1H, m, H_{glc}-2), 3.43,(1H, m, H_{glc}-3), 3.50 (1H, m, H_{glc} 4), 3.58 (1H, m, H_{glc}-5), 3.30 (2H, m, H_{glc} -6), 4.51 (1H, br, H_{Rha}-1), 3.10 (1H, m, H_{Rha}-2), 3.43 (1H, m, H_{Rha}-3), 3.54 (1H, m, H_{Rha}-4), 3.31 (1H, m, H_{Rha}-5), 1.17 (3H, d, H_{Rha}-6). ^{13}C NMR ($CDCl_3$): δ 158.1 (C-2), 135.1 (C-3), 180.0 (C-4), 160.0 (C-5), 100.0(C-6), 166.1 (C-7), 95.1 (C-8), 163.0 (C-9), 104.7 (C-10), 123.0 (C-1'), 118.2 (C-2'), 146.1 (C-3'), 150.1 (C-4'), 115.8 (C-5'), 123.4 (C-6'), 105.0 (C_{Glc}-1), 75.6 (C_{Glc}-2), 77.8 (C_{Glc}-3), 74.9 (C_{Glc}-4), 77.2 (C_{Glc}-5), 69.0 (C_{Glc}-6), 103.1 (C_{Rha}-1), 71.2 (C_{Rha}-2), 72.1 (C_{Rha}-3), 74.1 (C_{Rha}-4), 70.4 (C_{Rha}-5), 19.2 (C_{Rha}-6).

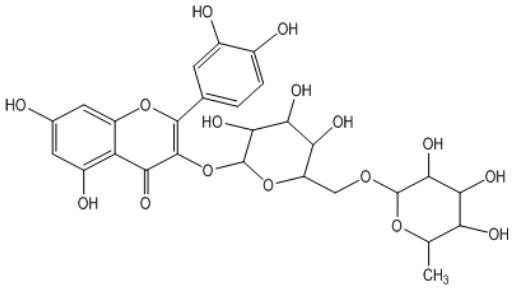

Figure 3: Quercetin -rhamnopyranosyl-D-glucopyranose.

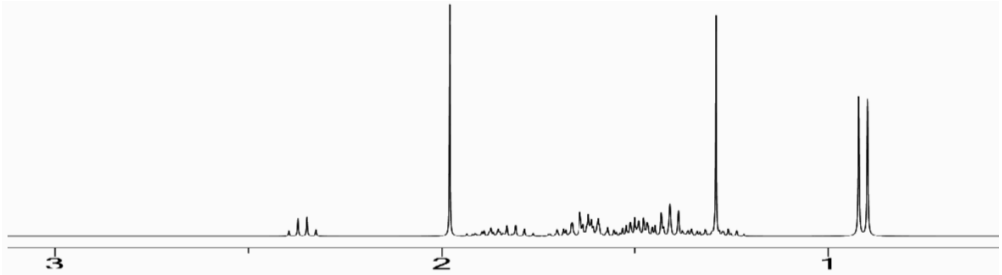

Figure 4: H^1 NMR of 10α- hydroxyoplopan-4-one (1).

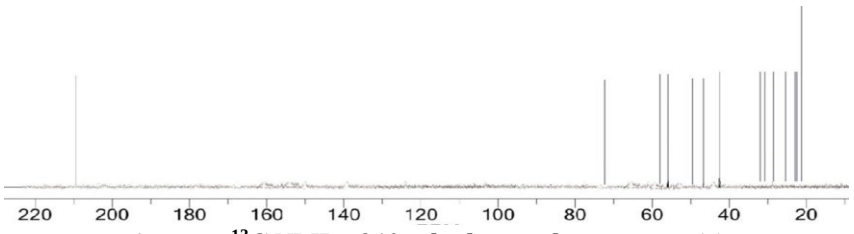

Figure 5: ^{13}C NMR of 10α- hydroxyoplopan-4-one (1).

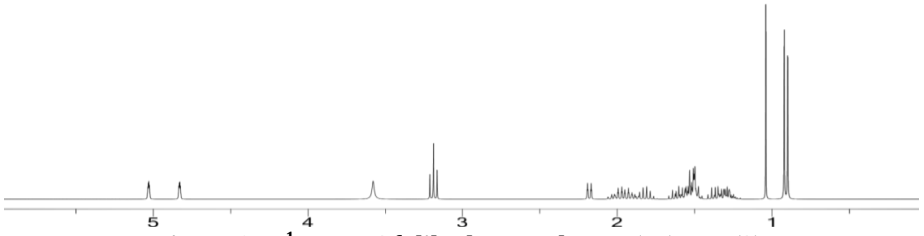

Figure 6: H^1 NMR Of dihydroxyeudesm-4(15)-ene (2).

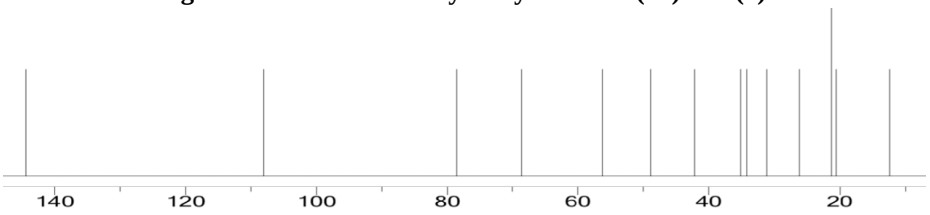

Figure 7: ^{13}C NMR of dihydroxyeudesm-4(15)-ene (2).

RESULTS AND DISCUSSION

Compound 1: The ^1H NMR showed one multiplet proton at δH 2.75 (1H, m), 5.46, High intensity Peaks at δ 2.30, 1.50, 1.10 and 0.88 are corresponding to methyl groups (Me- (15, 13, 14 and 12). 4 methyl, 4 methylene, 5 methine and 2 quaternary carbons presence in ^{13}C NMR spectrum . Carboxylic group signals become visible at δ 209.5. In addition of β-hydroxyl group to C8 is visible from a peak at δ 73.1. hydroxyoplopan-4-one, it has never been isolated before from *Caralluma quadrangula*, it reported from *Cassia buds*[21].

Compound 2: The 1H NMR showed signals for three angular methyl singlet's at δH 0.95, 0.85 and 0.71. proton of H-6 and H-1 appeared at δ 3.79 and 3.42. Olefinic protons present at δ 5.10 and 4.95 for H-15. 13C NMR showed fifty carbon signal including three CH3, five CH2, five CH and two quaternary carbons. The double bond carbons appeared at δ 147.4 and 108.1. The significant signal for the 1β, 6α-dihydroxyeudesm-4(15)-ene would be the signals for two carbon attached to hydroxyl group, which is C-1 and C-6 that appeared at δ79.2 and 67.8[22,23].

Compound 3: The ^1H NMR spectrum exhibited signals which were typical of a flavone compound. In addition to the presence of five aromatic protons; one was represented by two *meta*-coupled protons at *δ*H 6.23 (*d*, H-6) and 6.42 (*d*, H-8). ^{13}C NMR experiments showed one methyl, 15 methines, one methylene and 10 quaternary carbon atoms, one being the flavone carbonyl (C 180.0)[24]. NMR spectral data confirmed the sugar part assigned as glucose and rhamnose. A significant downfield shift of the methylene carbon appearing at C 69.1 and assigned to C-6 of glucose, indicated a (1 to 6) type of interglycosidic linkage to the rhamnose moiety. Qercetin-rhamnopyranosyl-D-glucopyranose isolated from *C.* quadrangula for first time , it was reported in many plants as *Taverniera aegyptiaca*[25,26].

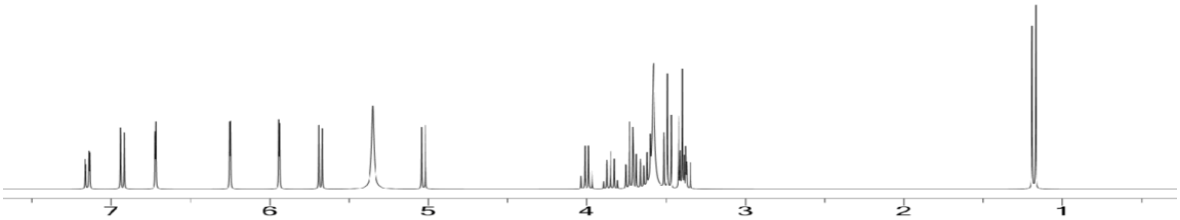

Figure 8: H^1 NMR of quercetin -rhamnopyranosyl-D-glucopyranose (3).

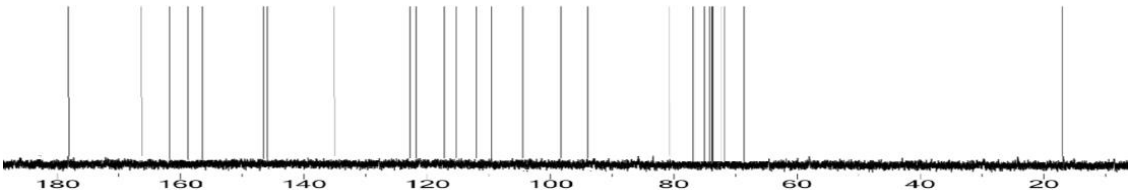

Figure 9: 13C NMR of quercetin -rhamnopyranosyl-D-glucopyranose (3).

CONCLUSION

The isolation and identification 10α- hydroxyoplopan-4-one, dihydroxyeudesm-4(15)-ene, and quercetin-rhamnopyranosyl- D-glucopyranose (Rutin), from the roots of *C. quadrangula*. The work was carried out by means of various physical (solvent extraction, column chromatography, and melting points) and spectral techniques.

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AUTHOR'S CONTRIBUTION

Al-Mahweety JAN: writing original draft, methodology. **Al-Fadaly A:** investigation, conceptualization. **Ahmed WA:** formal analysis, conceptualization. All authors revised the article and approved the final version.

DATA AVAILABILITY

Data will be made available on reasonable request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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