Photochemical Purification of root part of the medicinal herb, *C. quadrangular*

Abstract:-

Objective: To purification 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 & 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), Stigmasterol (5.0 mg) & quercetin- rhamnopyranosyl--D-glucopyranose (Rutin). (7.0 mg).

Conclusions: From this study, it can be concluded that the species found four pure compounds from C. *quadrangula.*

Keyword: *Caralluma quadrangula*, hydroxyoplopan-4-one, dihydroxyeudesm-4(15)-ene, Stigmasterol & quercetin- rhamnopyranosyl- D-glucopyranose (Rutin).

Introduction:

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). Anumber of Caralluma species use as anti-hyperglycemic activity of their crude extracts or their equivalent fractions (3-4).

The chemical and biological investigation of the members of genus Caralluma (3, 5) the anti-hyperglycemic activity of the extracts, fractions and the major pregnane glycoside of the aerial parts of C. quadrangula original from Kingdom of Saudi Arabia was investigated. The extract of C. quadrangula use as traditional medicine in Saudi, for thirst, hunger & for the treatment of freckles, diabetes, vitiligo & melasma (5-6). Several countries the species of *Caralluma* are fit to be eaten and variety division for the traditional medicine organization (7). Usually can be use as folk medicine as remedies to treat large multiplicity of diseases and health situation (8). In United Arab of Emirates *the species of C. arabica* use as traditionally for an emollient and diuretic. Also used to care for diabetes, hypertension & liver diseases. 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 (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 phytochemical constituents of *Caralluma* are pregnane glycosides, flavone glycosides, megastigmane glycosides, bitter principles, triterpenes & saponins (13, 14, 15, 16).

Materials and Methods:-

A.. General experimental procedures:-

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

B.. Plant material:-

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

C.. Extraction and Isolation:-

Shade dried roots were crushed & 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 & 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.

C..1. General extraction and isolation:-

The powder (2 Kg) of C. quadrangula roots was extracted (Soxhlet) with solvents (3X, 8 hours each) and the combined extracts evaporated to give a brown gummy residue (8 g), than separation & purified by silica gel flash column chromatography (FCC) with $CHCL_3$ containing increasing percentages of MeOH as eluent & collected 20 ml for each fraction. Fractions 3-10 were combined & rechromatographed by C.C. to yielded JA1 (4.5 mg) identified as 10α- hydroxyoplopan-4-one (1), JA3 (5.0 mg) identified as 1β, 6α-dihydroxyeudesm-4(15)-ene (2), *JA4 (5.0 mg)* identified as Stigmasterol (3) & JA4 (7.0 mg) identified as quercetin-rhamnopyranosyl- D-glucopyranose (Rutin). (4). NMR data used to identified for each pure compounds.

10α- hydroxyoplopan-4-one (1).

^{1}H-NMR (100 MHz, CDCl3) 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), 0.88 (3H, d, H-12); ^{13}CNMR (MHz, CDCl3) δ: 209.5 (C-14), 73.1 (C-8), 56.0 (C-3), 54.7 (C-9), 48.4 (C-5), 45.7 (C-4), 41.1 (C-7), 28.5 (C-10), 27.5 (C-1), 24.3 (C-2), 21.9 (C-6), 21.1 (C-11), 19.3 (C-13), 18.6 (C-15), 14.6 (C-12).

Fig.1. ***10α- hydroxyoplopan-4-one (1).***

1β, 6α-dihydroxyeudesm-4(15)-ene (2). ^{1}H-NMR (100 MHz, CDCl3) δ: 5.10 (1H, brs, H-15), 4.95 (1H, brs, H-15), 3.79 (1H, t, H-6β), 3.42 (1H, dd, H-1α), 2.33 (1H, ddd, J = 2.0, 5.0, 13.0 Hz, H-3α), 2.24 (1H, sept, J = 2.0, 6.5 Hz, H-11), 2.07 (1H, ddd, J = 5.0, 13.0, 13.0 Hz, H-3β), 1.91 (1H, s, H-8), 1.85 (1H, ddd, J = 2.0, 4.0, 12.0 Hz, H-2α), 1.75 (1H, brd, J = 9.5 Hz, H-5α), 1.53 (1H, m, H-2β), 1.53 (1H, m, H-8), 1.43 (1H, brs, 1-OH), 1.27 (1H, m, H-7α), 1.19 (1H, m, H-9a), 1.17 (1H, m, H-9b), 0.95 (3H, d, H-13), 0.87 (3H, d, H-12), 0.71 (3H, s, H-14) ; ^{13}C-NMR (MHz, CDCl3) δ: 147.4 (C-4), 108.1 (C-15), 79.2 (C-1), 67.8 (C-6), 56.4 (C-5), 49.9 (C-7), 42.2 (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).

Fig.2. dihydroxyeudesm-4(15)-ene

Stigmasterol (3). ^{1}H NMR (100 MHz, CDCl3): δ 0.84 (10H, q, J=7.19 Hz, Me-21, Me-26, Me-27, Me-29), 0.93 (1H, t, J=5.40 Hz Me-21β), 5.32 (1H, d, J=4.62 Hz, H-22), 1.00 (8H, d, J=6.57 Hz , H-1α, 1β, Me-18, Me-19β, Me-19γ, Me-21α), 1.21 (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,), 5.18

(1H, q, J=7.86 Hz, H-23), 1.50 (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), 1.83 (2H, d, J=10.05 Hz, H7α, 7β), 2.11 (3H, m, J=8.07 Hz, 3-OH, H-20, H-24), 2.26 (2H, t, J=8.13 Hz, H-4α, 4β), 3.49 (1H, m, J=5.09 Hz H-3), 5.01 (1H, q, J=7.85 Hz H-6). ^{13}C NMR (CDCl3): δ 12.2 (C-29), 12.2 (C-21), 19.1 (C-27), 20.5 (C-26), 21.3 (C-19), 21.4 (C-18), 21.5 (C-11), 24.5 (C-16), 25.5 (C-15), 29.0 (C-28), 31.8 (C-8), 32.0 (C-7), 32.3 (C-25), 33.1 (C-12), 37.1 (C-1), 37.4 (C-20), 40.1 (C-2), 41.2 (C-10), 42.1 (C-13), 42.4 (C-9), 50.3 (C-4), 51.36 (C-14), 56.1 (C-24), 57.1 (C-17), 71.9 (C-3), 122.0 (C-6), 129.4 (C-22), 138.0 (C-23), 141.1 (C-5).

Fig.3. Stigmasterol

quercetin- L-rhamnopyranosyl- (1→6)-D-glucopyranose (4). ^{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), 4.50 (1H, br, H_{Rha}-1), 3.09 (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), 135.3 (C-3), 179.8 (C-4), 160.0 (C-5), 99.7 (C-6), 165.8 (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), 77.3 (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).

Fig.4. quercetin -rhamnopyranosyl-D-glucopyranose

Result and Discussion:-

Compound (1). *The ^{1}H NMR showed one multiplet proton at δH 2.75 (1H, m), 5.46, High intensity Peaks at δ 2.30, 1.50, 1.10 & 0.88 are corresponding to methyl groups (Me- (15, 13, 14 & 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.

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 & 4.95 for H-15. 13C NMR showed fifty carbon signal including three CH3, five CH2, five CH& 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 & 67.8.

Compound (3). ^{1}H NMR spectrum showed two signals corresponding to olefinic region were experimental with high chemical shifts values. A multiple at δ 3.52 is typical to a carbinylic proton of sterol moiety. Peaks at δ 5.32 , 5.18 & 5.01, in low value correspond to ethylene protons respectively present on C22, C23 and C6. Peaks confirm at δ 1.00 & 0.84, are resulting to methyl groups (Me-19, 18, 26, 27 & 29). ^{13}C NMR spectrum shows the presence of 6 CH_3, 9 CH_2, 11 CH & 3 quaternary carbons. Signals of double bond appeared at δ 141.1 & 122.0. Attachment of β-hydroxyl group to C3 is visible from a peak at δ 71.9. High intensity peak at δ 21.3 & 21.4 represent angular methyl carbons – C19 and C18.

Compound (4). The ^{1}H 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, J* = 2.4 Hz, H-6) and 6.42 (*d, J* = 2.4 Hz, 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). 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 (1to 6) type of interglycosidic linkage to the rhamnose moiety.

Conclusion :-

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

Reference:-

1. Rajendra R., Kamala. USP filed. **2004**; 4: 6376657.

2. Mohammad D, M. Al-Khateeb, E. Riyadh, Al-Hashem F., Mohammad K Nabil. In vivo, acute, normo-hypoglycemic, antihyperglycemic, insulinotropic actions of orally administered ethanol extract of *Citrullus colocynthis* (L.) Schrab pulp. American Journal of Biochemistry and Biotechnology. 2009; 5(3):119-26.

3. K.P. Prabhakar , M. Doble, A target based therapeutic approach towards diabetes mellitus using medicinal plants, Curr. Diabetes Rev. 4 (2008) 291–308.

4. Sattar E. A., F.M.H. Harraz, Ghareib Elberryl S.A., A.A., S. Gabr, M.I. Suliaman, Antihyperglycemic and hypolipidaemic effects of the methanolic extract of Caralluma tuberculata in streptozotocin-induced diabetic rats, Nat. Prod. Res. 25 (2011) 1171–1179.

5. Abdel-Sattar E., Abdel-Naim A. B., Khedr A., Shehata I.A., Anti-hyperglycemic activity of Caralluma tuberculata in streptozotocin-induced diabetic rats, Food Chem. Toxicol. 59 (2013) 111–117.

6. Habibuddin M., . Daghriri H.A, Humaira T., Al Qahtani M.S., Hefzi A.A.H., Anti-diabetic effect of alcoholic extract of Caralluma sinaica L. on streptozotocin-induced diabetic rabbits, J. Ethnopharmacol. 117 (2008) 215–220.

7. Latha S., Rajaram K.., Suresh K.P. Hepatoprotective and antidiabetic effect of methanol extract of Caralluma fimbriata in streptatozocin induced diabetic albino rats, Inter. J. Pharm. Pharmaceut. Sci. 6 (2014) 665–668.

8. Gushash A.S. Plants in the Mountains of Sarat and Hejaz. Sarawat, Al Madinah press, KSA, 2006.

9. Abdel-Sattar E., Ahmed A. A., M. E. Hegazy, M. A. Farag, M. A. Al-Yahya. Acylated pregnane glycosides from Caralluma russeliana. Phytochemistry **2007**; 68, 1459- 1463. 211.

10. Oyama M., Iliya I., Tanaka T., Linuma M. Five new steroidal glycosides from Caralluma dalzielii. Helvetica Chimica Acta **2007**; 90, 63-71.

11. Ahmad M. M., Qureshi S., Shah A., N. S. Qazi, R. M. Rao, A. M. Al-Bekairi. Anti-Inflammatory activity of Caralluma tuberculata alcoholic extract. Fitoterapia **1983**; 46, 357-360.

12. Zakaria M. N., Islam M. W., Radhakrishnan R., Chen H. B., KamiM. l, A. N. Al-Gifri, K. Chan, Al-Attas A.. Anti-nociceptive and anti-inflammatory properties of Caralluma arabica. J. Ethnopharmacol **2001;** 76, 155-158.

13. Ramesh, Y. N. Rao, A. V. Rao, M. C. Prabhakar, C. S. Rao, N. Muralidhar, B. M. Reddy. Antinociceptive and anti-inflammatory activity of a flavonoid isolated from Caralluma attenuata. J. Ethnopharmacol **1998**; 62, 63-66.

14. V. M. Dembitsky. Chemistry and biodiversity of the biologically active natural glycosides. Chemistry & Biodiversity **2004**; 1, 673-781.

15. D. S. Kumar. A medicinal plants survey for treatment of obesity. Journal of Pharmacy Research **2011;** 4, 597-600.

16. A. Bader, A.Braca, N. De Tommasi, I. Morelli. Further constituents from Caralluma negevensis. Phytochemistry **2003**; 62, 1277-1281.

17. A. Braca, A. Bader, I. Morelli, R. Carpato, G. Urchi, C. Izza, N. Tommasi. New pregnane glycosides from Caralluma negevensis. Tetrahedron **2002;** 58, 5837-5848.

18. Malladi , Ratnakaram , Suresh Babu & Pullaiah. Phytochemical Investigation of C. lasiantha: Isolation of Stigmasterol, an Active Immunomodulatory Agent. International Journal of Chemistry Sciences **2017**; 15, 339-407.

19. Trease GE, Evans WC. Pharmacognosy, Saunders. Elsevier, Amsterdam, The Netherlands **2002**;36: p.51.

20. Gupta AK. Introduction to Pharmaceutics-1. CBS publication, New Delhi 2004.