

A Novel Pregnane Glycoside From The *Caralluma Umbellata* Haw (*Asclepiadaceae*) Roots

Kalyani.K, Anuradha.V* & Jayalakshmi.G

Department of Chemistry R.V.R&J.C College of Engineering, Guntur, Andhra Pradesh, India.

*Department of BS & H, Vignan's Nirula Institute of Technology and Science, Guntur, A.P., India.

Department of Chemistry Vignan Degree College Palakaluru, Guntur, Andhra Pradesh, India.

*Email of the corresponding author: var_chemistry@rediffmail.com

Abstract:

From the roots of *Caralluma umbellata* belonging to the family *Asclepiadaceae*, a novel pregnane glycoside was isolated. Its structure was elucidated 3-O- β -D-glucopyronosyl, 8 β -isopropoxy, 14 β -ethynyl, pregn-5en-21,21 dimethyl 16,17 lactone on the basis of spectroscopic data. This is the first report from the genus *Caralluma* as well as its family *Asclepiadaceae*.

Keywords: *Caralluma umbellata* roots, *Asclepiadaceae*, pregnane glycoside, extraction, isolation, spectral studies.

Introduction:

Caralluma umbellata (Haw) is a succulent perennial herb grows up to 30 cm tall in dry and arid regions of Tamilnadu, Orissa, Andhra Pradesh, Karnataka. The genus *Caralluma* (*Asclepiadaceae*) is known to be a rich source of pregnanes¹⁻⁷, pregnane glycosides^{3,8-27}, flavanoidal glycosides²⁸⁻³⁰, megastigmane glycosides³⁰, triterpenoids^{27,31,32}. These pregnane glycosides were reported to be cytotoxic in the recent years¹². In this paper we report isolation and characterization of a novel pregnane glycoside from the roots of *Caralluma umbellata*.

Experimental:

The plant material of *Caralluma umbellata* was collected from Tirumala hills, Tirupathi in December 2009. Plant material was identified by using regional flora conserved at herbarium, Dept of Botany Sri Venkateswara University. All chemicals used in this experimental work were AR grade sold from NSP Guntur, purified according to the standard procedures, methanolic sulfuric acid (98:2) is used as spraying reagent.

Instruments used:

Melting point was determined on a Buchi capillary melting point apparatus. The ¹H NMR was recorded in CDCl₃ solution by using 400 MHz spectrometer. IR spectra was recorded on FT-IR spectrometer. MS spectra was recorded on agilent 6430 series triplet Quad MS spectrometer.

Extraction and isolation:

The roots of the plant *Caralluma umbellata* (Haw) about 3kgs were extracted with ethanol in a soxhlet apparatus. The extract (about 150g) was impregnated on minimum amount of silica gel and washed successively with Hexane, Benzene and Methanol. Benzene and Methanol washes were impregnated on minimum amount of silica gel individually and washed with Hexane, Ether, Acetone and finally with Methanol.

The ether eluates of Benzene and Methanol extract have shown the similar spotting on TLC. Concentrated (10gms) and subjected to repeated column chromatography with Hexane, Ethylacetate and Methanol. Fractions of about 200 ml were collected and examined on TLC using different solvent systems, spraying with methanolic sulphuric acid (98:2). Fractions showing similar spot pattern were grouped and worked individually. The fractions 20&21 eluted using Hexane and Ethylacetate (9:1) has shown one single blue spot with R_f value 0.42 in pure Benzene solvent system. This on recrystallisation with methanol separated as white crystalline solid, gave a positive Liebermann-Burchard reaction showing the presence of a steroid.

Yield:16 mg m.p:273-275 °C

Spectral studies :

I.R.(ν_{\max}) cm^{-1}	:1793, 1601, 2253, 2869, 2931.
$^1\text{H NMR}(\text{CDCl}_3)$	1.05 - 1.03 [2H, m, 1 β ,1 α],
δ (ppm)	1.254 [2H, m, 2 α , 2 β], 2.279 [1H,m,3 α], 5.12-5.26[anomeric proton m, H-1'], 1.573-1.593 [2H,m,4 α ,4 β], 5.35 [1H,m,H-6], 1.497-1.477 [2H,m,7 β ,7 α], 1.64[H,m,H-9 α], 2.0-1.75[2H,m, 11 α ,11 β] 1.72-1.71[2H,m,H-12 α ,12 β],1.18- 1.17 [2H,m,15 α ,15 β], 1.161[1H, m, H-16], 1.51[1H,m, H-17], 0.75 [6H, S, H-18, 19], 0.87 [3H,m H-22], 0.95[3H,m,H-23], 3.81[1H,septetH-24], 1.23 [6H, d, H-25, 26], 2.1[1H, m, H-28]
Mass(ESIMS)	: 604,559, 577, 397, 382, 354,329, 301

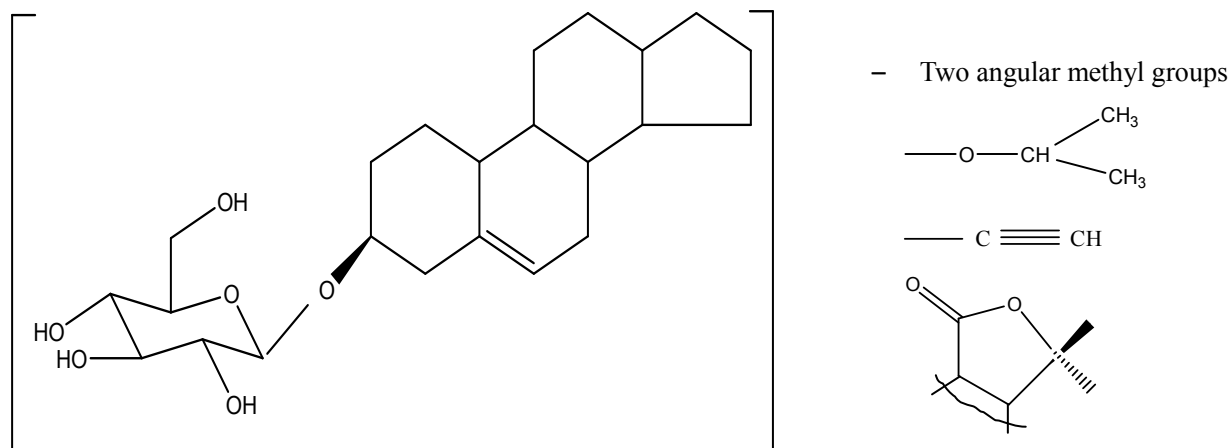
Results and discussion

Compound obtained as white crystalline solid with m.p (273-275°C) analysed for $\text{C}_{34}\text{H}_{50}\text{O}_9$, showed positive test for Liebermann-burchard reaction indicating it to be a steroid. The m/z 604.5 indicated [M+2] peak, m/z at 397.4 [$\text{M}^+ - \text{C}_6\text{H}_{11}\text{O}_5 + \text{H}$] indicated that the compound contains one sugar unit. A clear septet in $^1\text{H NMR}$ at δ 3.55 indicated the presence of isopropyl ether, which is further confirmed by a doublet at δ 1.23 corresponding to six hydrogens.

IR stretching frequency at ν_{\max} at 1793 cm^{-1} indicated the presence of a lactone ring. Absence of any other downfield signal indicated that the ring is substituted. The stretching at 2253 cm^{-1} and an acetelinic proton at δ 2.1 corresponding to [1H] indicated the presence of mono substituted acetelinic linkage. The $^1\text{H NMR}$ spectrum showed a doublet at δ 5.12 to 5.26 integrating for one proton indicated that it has one mono saccharide moiety with the glycosidic linkage in β -configuration. The other hydrogens of the glycoside are not clearly visible in the spectrum very weak signals between δ 3.7 to 4.2 might be the other hydrogens of glycoside. This is further confirmed by a down field signal at δ 2.29[1H, m] indicated that the glycosidic bond is β -glycosidic^{11,17} with the above signal corresponding to H-3 α . This is also supported from literature, by the isolation of caumbelloside-II from *Caralluma umbellata*⁴ shows similar spectral data for glycoside ring.

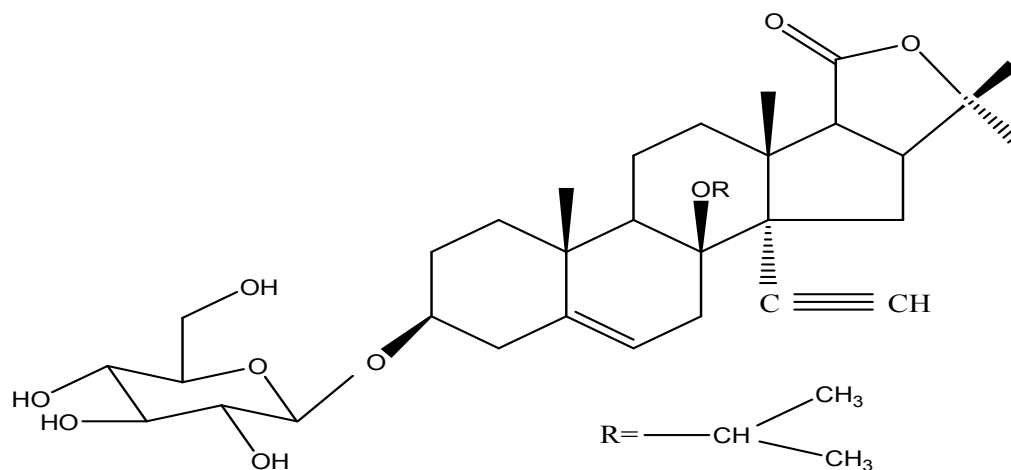
One proton multiplet δ 5.35 indicated the presence of trisubstituted olefinic double bond. This olefinic double bond is assigned to 5,6 positions based on biogenic criteria^{4,7,15}. $^1\text{H NMR}$ signals at δ 1.57-1.59[2H,m],1.47-1.49[2H,m] corresponds to allylic 4 α ,4 β and 7 α ,7 β respectively. The NMR spectra showed the presence of signals at δ 0.87[3H] and 0.95[3H]. The down field broad singlets corresponds to 0.87 and 0.95 respectively showed the presence of gem dimethyl groups in the lactone ring. The six hydrogen singlet at δ 0.75 indicated the presence of two angular methyl groups.

Therefore the partial structure of compound can be given as



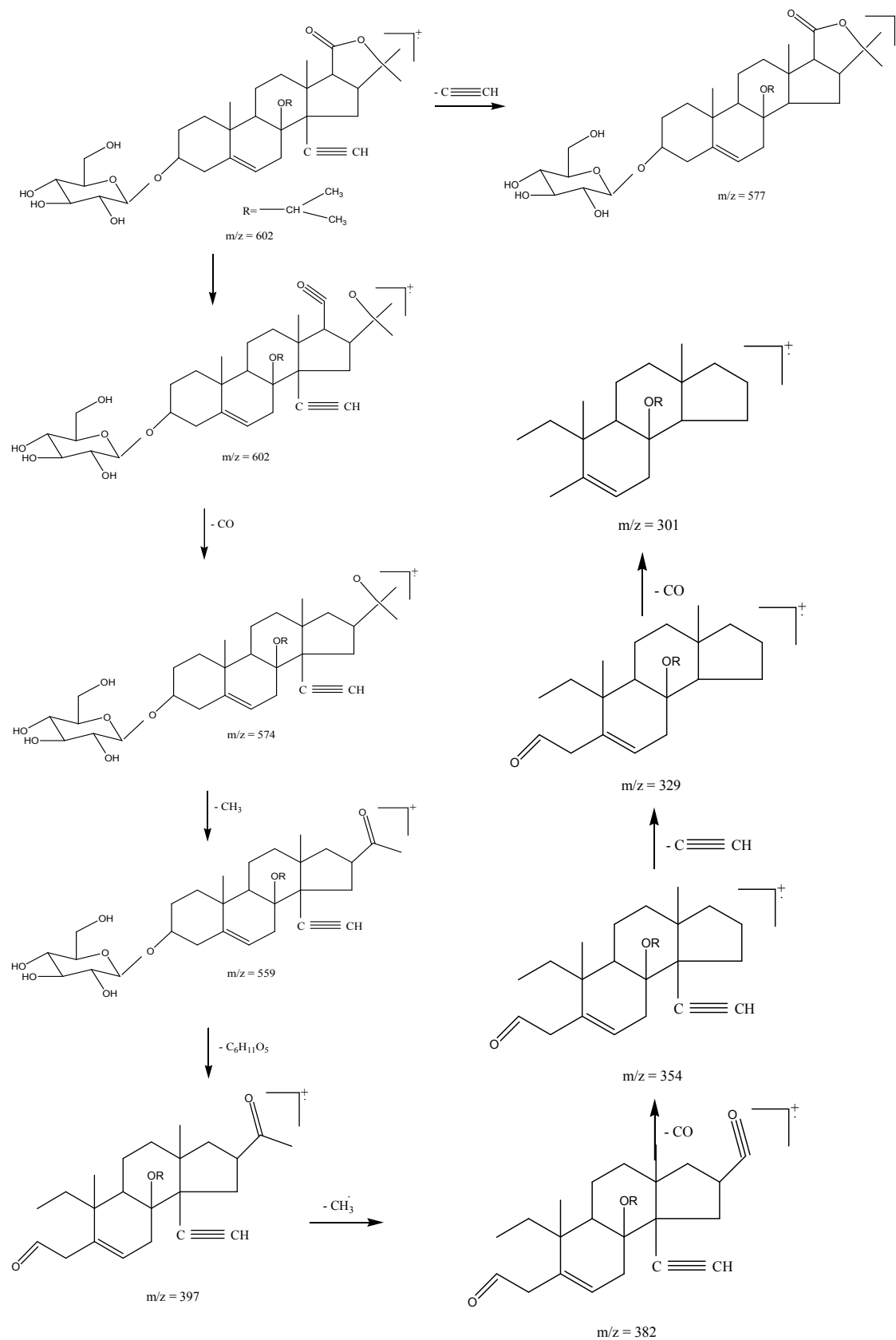
The two angular methyl groups are assigned 10,13th carbons based on biogenetic criteria. A one proton clear septet at δ 3.81 indicated the presence of isopropyl ether group and absence of any other down field signals in the ring protons indicated this group to be attached to a tertiary carbon. Thus isopropyl ether group is allocated to C-8, this substitution also supported from the literature ^{21,23}.

A one proton multiplet at δ 1.51 and another one proton multiplet at δ 1.16 indicated the presence of lactone ring at H-16, 17 positions. A two proton multiplet at δ 1.18-1.17 is allocated to H-15 α ,15 β with the acetylinic group at C-14. Thus the structure of compound is established as

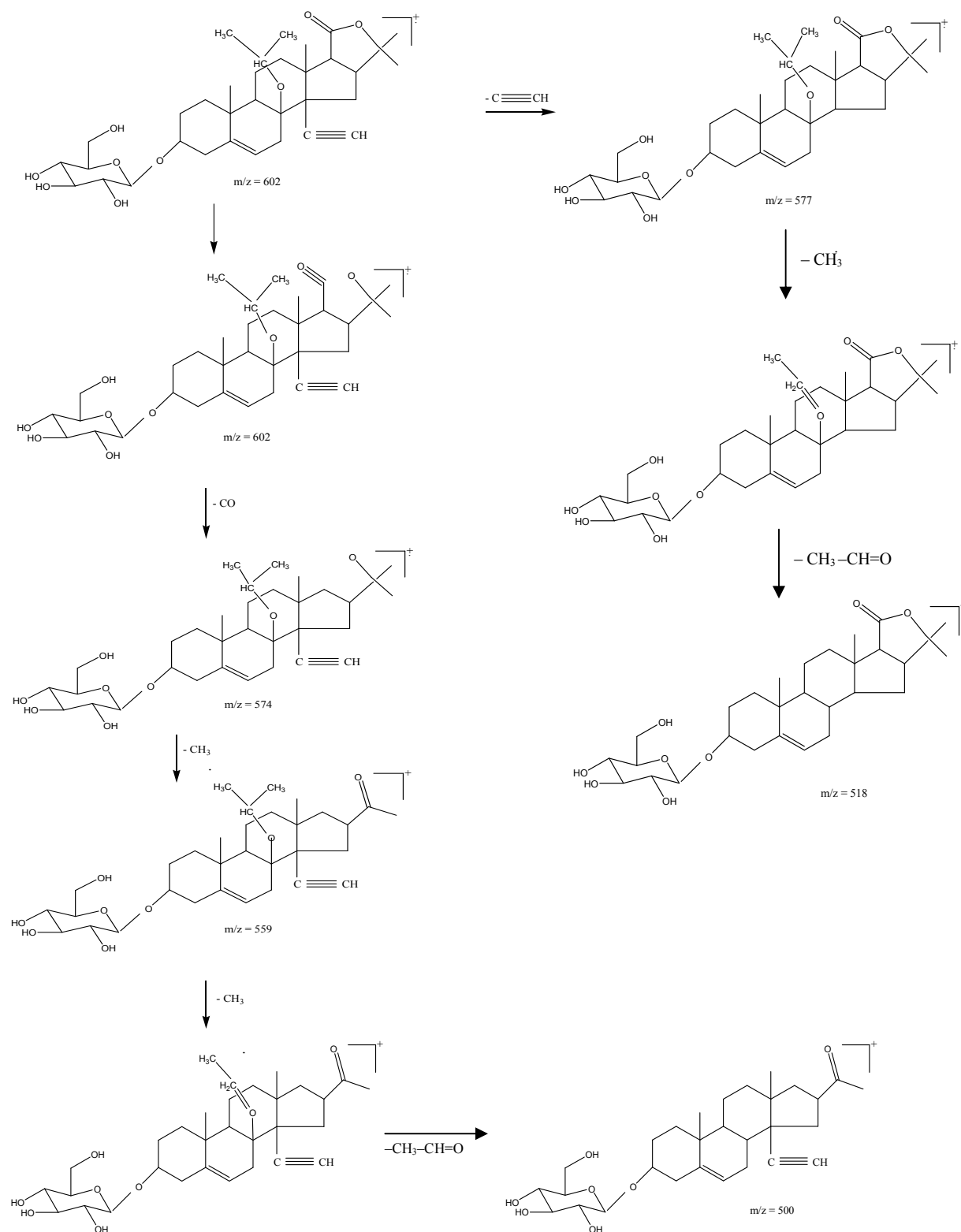


The structure was further supported by MS spectral data.

Mass fragmentation pattern-1 of compound



Mass fragmentation pattern – 2 of compound



Conclusion:

The compound was assigned as 3-O- β -D-glucopyronosyl, 8 β -isopropoxy, 14 β -ethynyl, pregn-5en-21, 21 dimethyl, 17 lactone. This is the first report from the genus *Caralluma* as well as family *Asclepiadaceae*.

Acknowledgements:

The author wish to express sincere thanks to the management of R.V.R & J.C College of Engineering for their support and management of Vignan institutions for the facilities provided for pursuing research.

References

1. Rudolf, T. and Gunter, M., *Tetrahedron Letters*, (1967) 1359-1363.
2. Nikadio, H., Yuzuru, S., Hiroshi, M., *Chem. Pharm. Bull.* (1967) **15(5)**, 725-726.
3. Olaf Kunert, Nebojsa Simil, Earla Ravinder, Bobbala Ravi Kumar, Robert Michael Alex et.al., *Phytochemistry Letters*, (2009) **2(3)**, 134-135.
4. Lin, L.J., Lin, L., Gil, R.R., Cordell, G.A.Ramesh, M., Srilatha, B., Reddy, B.M and Appa Rao, A.V.N., *Phytochemistry* (1994) **35(6)**, 1549-1553.
5. Khalil, A.T., *Flitoterapia* (1995), **66(3)**, 261-264.
6. K.Suresh Babu, Vidadala Rama Subba Rao and Sri Vedavyasa Radhakrishnan et.al. *Journal of Asian Natural products research* (2008), **10(11)**, 1013-1016.
7. Medikodu Kishore, K.Surendra Babu, Y.Hanumantha Rao, G.Nagahima Bindu and M.Janardhan, *International Journal of applied biology and pharmaceutical technology* (2010) **1(2)** 545-549.
8. Hayashi, K., Iida, I., Nakao, Y. and Kaneko, K., *Phytochemistry* (1998), **27(2)**, 3919-3922.
9. Tanaka, T., Tsukamoto, S., and Hayashi, K., *Phytochemistry* (1990), **29(1)**, 229-237.
10. Ghazalah H.R.Zwani, K.Usmanghani, Mansoor Ahmed, Vigar Uddin Ahmad, *Natural Products Letters* (1993) **2(2)**, 97-104.
11. Essam Abdel Sattar, Fathalla M. Horraz et.al., *Phytochemistry* (2008), **69**, 2180-2186.
12. Abdul Waheed, James Barker, Stephen J.Barton, et.al., *Journal of Ethnopharmacology* (2011).
13. Abdelsattar, E., Al-Yahya M.A.A., Nakamura, N., et.al., *Phytochemistry* (2001) **57**, 1213-1217.
14. Abdel Sattar.E., Meselhey,R.M.,Al-Yahya, M.A.A.,*Planta Med.*(2002) **68**, 430-434.
15. Qiu, S.X., Cordella, G.A., Ramesh, M., Ravi Kumar. B., Radha Krishna M., Mohan G.K., et.al., *Phytochemistry*, (1999) **50**, 485-491.
16. Al-Yahya, M.A.A., Abdel-Sattar, E and Guittet, E., *J.Nat. Prod.* (2000) **63**, 1451-1453.
17. F.Hegazy, Mohammed A. Farag and Mohammed Abdul Aziz, Al-Yahya, *Phytochemistry* (2007) **68(10)**, 1459-1463.
18. Reddy B.M., and Appa Rao, A.V.N., *Phytochemistry* (1994) **35(6)**, 1549-1553.
19. Radha Krishna, M., Krishna Mohan, G., Reddy, B.M.Rao, N.Y., Srinivas, B., Thomas, N.S. and Appa Rao, A.V.N., *Phytochemistry* (1997) **46(2)**, 333-340.
20. Halim, A.F. and Khalily, A.T., *Phytochemistry*, (1996) **42(4)**, 1135-1139.
21. Fathi T. Halaweish, Eric Huntimer and Ashaf T. Khalil, *Photochemical Analysis* (2004), **15**, 189-194.
22. Braca. A., Bader. A., Morelli. I., Scarpata. R., Turchi.G., Pizza.C and Tommsi.N, *Tetrahedron* (2002) **58**, 5837-5842.
23. Olaf Kunert, Belvotagi Venkatrao Adavi Rao, Gummadi Sridhar Michael Alex, Wolfgang Schuhly – *Helvetica Chimica Acta* (2006) **89**, 201-209.
24. Marinella De Leo, Nunziatina De Tommasi, Rokia Sanogo, Giuseppina Autore, Stefania Marzocca, Cosimo Pizza, et al., *Alessandra Braca* (2005) **70**, 573-585.

25. Olaf Kurnet Vijaya Lakshmi Gurunath Rao, Gumohadi Sridhar Babu, Palatheeya Sujatha, Malayalam Sivagamy, Sandala Anuradha, Bobbala Ravi Kumar, *Chemistry & Biodiversity*, (2008) **5**. 239-249_.
26. G.H.Rizwani, Mansoor Ahmad, V.U Ahmad K. Usmanghani *Spectroscopy Letters* (1993) **26(8)**, 1427.
27. Chazala Hafeez Rizwani, Caralluma Tuberculata and Caralluma edulis. Ph.D Thesis submitted to University of Karachi, Department of Pharmacology (1991).
28. Rizwani, G.H., Ahmad, M., Usmanghani, K., and Ahmad, A.U., *Pakistan Journal of pharmaceutical Sciences* (1990) **3(2)**, 27-32.
29. S.Chan, K., Habibullah, A.F., Ahmad, F., Gunasekhar, C., Samuel, *J.Pharm. Pharmacol.*, (2000) **52**, 265.
30. Bader, A., Braca, A., De Tommasi, N., Morelli, I., *Phytochemistry* (2003) **62(8)**, 1277-1281.
31. Castro Reyes, Victor A, Francisco, Cosme.G., Gonzalez, Antonio G., Hernandez, Rosendo Surat, Emesto, *Phytochemistry* (1980) **19(10)**, 2210-2212.
32. G.Jaya Lakshmi, M.Phil Thesis submitted to Acharya Nagarjuna University (2008).

This academic article was published by The International Institute for Science, Technology and Education (IISTE). The IISTE is a pioneer in the Open Access Publishing service based in the U.S. and Europe. The aim of the institute is Accelerating Global Knowledge Sharing.

More information about the publisher can be found in the IISTE's homepage:

<http://www.iiste.org>

CALL FOR PAPERS

The IISTE is currently hosting more than 30 peer-reviewed academic journals and collaborating with academic institutions around the world. There's no deadline for submission. **Prospective authors of IISTE journals can find the submission instruction on the following page:** <http://www.iiste.org/Journals/>

The IISTE editorial team promises to review and publish all the qualified submissions in a **fast** manner. All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Printed version of the journals is also available upon request of readers and authors.

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digital Library, NewJour, Google Scholar

