A peer-reviewed journal
PSM Biological Research
2016 | Volume 1 | Issue 2 | 66-69
ISSN: 2517-9586 (Online)

www.psmpublishers.org

Short Communication **Open Access**

Chemical study on the leaves of *Cyphostemma digitatum*

Jamal A. N. Al- Mahweety[1*]

[1]School of Chemical Sciences, Sana'a University, Yemen.

Received: 03.Jun.2016; Accepted: 07.Jul.2016; Published Online: 24.Sep.2016
***Corresponding author: Jamal A. N. Al- Mahweety; Email: jamal.nasser2009@gmail.com**

Abstract
Phytochemical study on the leaves of *Cyphostemma digitatum* obtained from Sana'a has been conducted. The separations of the chemical components were carried out by different chromatography techniques and their structures were elucidated by spectroscopic methods such as mass spectrometry (MS), ^{1}H and ^{13}C NMR and by comparison with those of previously reported data. Four compounds were isolated and identified as follows, triterpenes which was identified as Lupeol, Dioxo-urs-12-en-28-oic acid and Longistylin A, Piceid as a stilbenoid glucoside.
Keywords: Leaves of *Cyphostemma digitatum*, Longistylin A, Lupeol, Dioxo-urs-12-en-28-oic acid and Piceid, NMR analysis.

To cite this article: Al- Mahweety, J.A.N., 2016. Chemical study on the leaves of *Cyphostemma digitatum*. PSM Biol. Res., 01(2): 66-69.

INTRODUCTION

The species *Cyphostemma digitatum* also known as "halka" is member of Vitaceae family. *Cyphostemma digitatum* has been traditionally used in Yemen for culinary and medical purposes (Al-Duais, 2009). Due to its increasing demand *C. digitatum* is exported to various parts of Yemen and other countries in the world (Al-Duais, 2009). The main uses of this plant are, as an ethnic medicine and food additive. The leaves and fleshy stem branches of *C. digitatum* were used for treatment of gastroenteritis, general weakness, fatigue, vomiting, nausea, malaria and headache (Al-Duais, 2009). Therefore, *C. digitatum* vanished from many parts of Yemen. The phenolic contents of *C. digitatum* were tested for high antioxidant activity by various methods (Al-Duais, 2009). It has been documented that this plant is a rich source of food ingredients and micronutrients, including vitamin C, vitamin E, provitamin A and carotenoids (Al-Duais *et al.,* 2009). The objective of this study was isolation and identification of chemical compounds from *Cyphostemma digitatum*.

MATERIALS AND METHODS

Collection of Plant Material

Leaves of *Cyphostemms digtatum* were assembled from various parts of Sana'a city. To keep the record a voucher specimen of YJN 001 for the plant was submitted at the Sana'a University Herbarium.

Extraction and Fractionation

The leaves of *Cyphostemms digtatum* were air-dried and grinded. The leaf powder (1000 g) was extracted (Soxhlet) using acetone (4X, 20 h each) and the obtained extracts were evaporated to yield a green residue (26 g). Further the extract was processed by silica gel flash column chromatography using hexane to increase percentage of ethyl acetate (EtOAc) as eluent and the yield of each fraction was 20 mL. Fraction 3 contained Longistylin A (15 mg), Fractions 5-9 (40 mg) were purified by reparative TLC with EtOAc - $CHCl_3$ (7:3) to yield Lupeol, (8.2 mg), Fractions 11 contained Dioxo-urs-12-en-28-oic acid, (4.3 mg) and fractions 6-7 comprised of Piceid (5.0 mg). Longistylin A, Lupeol, Dioxo-urs-12-en-28-oic acid and Piceid, were identified by correlating to the data from preceding NMR and mass spectra (Yadav, 2003; Kuete *et al.,* 2007; Chen *et al.,* 2000; Gollapudi *et al.,* 1989).

Longistylin A

Colorless crystals, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72(d, 7.5, H-2a & 6a), 7.45 (d, 7.4, H- 3a & 5a), 7.30 (dd, 7.3, 2, H-4a), 6.9 (d, 5.30, H- 7a &8a), 6.43 (s, H-14a), 6.38 (s, H-10a), 5.73 (t, 1.10, H-2'), 5.40 (OH), 3.86 (s, OCH_3),3.2 (d, .086, H- 1'), 2.1 (s, H- 4' & 5'). ^{13}C NMR (100 MHz, $CDCL_3$-D): δ 160.8 (C-11a), 157.1 (C-13a), 137.9 (C-9a), 137.4 (C-1a), 131.8 (C-3'), 130.1 (C- 2a & 6a), 129.3 (C- 3a & 5a), 128.2 (C- 4a), 126.9 (C- 7a), 125.8(C- 8a), 124.3 (C-2'), 113.6 (C-12a), 106.0 (C- 10a), 105.3 (C- 14a), 57.2 (OCH_3), 25.6 (C-5'), 23.1 (C- 1'), 19.8 (C-4') (Figure 1).

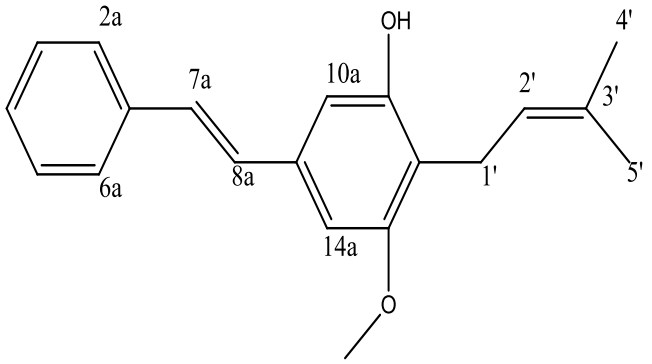

Fig. 1. Structure of Longistylin A

Lupeol

White powder, M.P. 215-216 ºC. EIMS for $C_{30}H_{50}O$ m/z (rel. int.): 426 (33.4%), 365 (14.5%), 207 (51.3%), 189 (25.8%), 161 (22.9%), 135 (71.0%), 107 (100%). ^{1}H NMR (400 MHz, $CDCL_3$-D): δ 4.70, 4,61 (s, H-29a, b),3.32 (dd, 4.81, 11.0, H-3), 0.74, 0.82, 0.85, 0.90, 1.01, 1.12, 1.35 (s, Me×7). ^{13}C NMR (100 MHz, CDCL3-D): δ 152.1 (C-20), 115.3 (C-29), 80.1 (C-3), 56.5 (C-5), 49.9 (C-9), 47.9 (C-18), 44.6 (C-19), 43.8(C-17), 42.7 (C-14), 40.9 (C-8), 39.8 (C-22), 39.0 (C-13), 37.9 (C-4), 37.4 (C-1), 36.3 (C-10), 35.7 (C-16), 32.9 (C-7), 31.0 (C-21), 28.4 (C-23), 26.6 (C-15), 26.2 (C-12), 24.9 (C-2), 22.1 (C-11), 20.5 (C-30), 19.3 (C-6), 18.7 (C-28), 17.3 (C-25), 16.4 (C-26), 14.9 (C-24), 14.1 (C-27) (Figure 2).

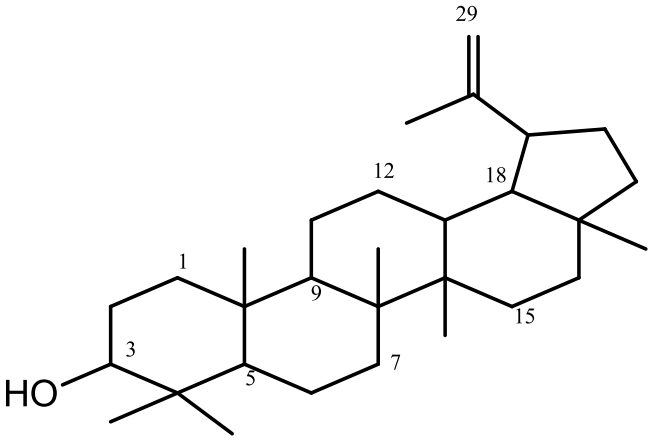

Fig. 1. Structure of Lupeol

Dioxo-urs-12-en-28-oic acid

White powder, MS (rel. int.): 468 , 454, 441, 440, 439, 424, 423, 407, 262, 260, 248 (100%), 203, 191, 189, 133, 120, 118, 106, 104. ^{1}HNMR (400 MHz, CDCl3): δ 0.87 (*s*, 26 Me), 0.91 (*d*, 6.5, 29 Me), 1.05 (*d*, 6.5, 30 Me), 1.13 (*s*, 25 Me), 1.21 (*s*, 23 Me), 1.32 (*s*, 27 Me), 2.19 (*d*, 11.0, H-18), 2.50 (*m*, H-2), 5.36 (*m*, H-12), 9.68 (*s*, -CHO). ^{13}C-NMR (CDCl): δ 17.6 (C-25), 17.9 (C-26), 18.2 (C-11), 20.5 (C-6), 21.9 (C-30), 22.3 (C-2), 23.1 (C-29), 24.7 (C-27, C-16), 28.6 (C-15), 29.3 (C-23), 31.8 (C-21), 33.6 (C-7), 34.9 (C-10), 36.7 (C-22), 37.3 (C- 4), 38.1 (C-20), 39.7 (C-8), 40.5 (C-1), 41.8 (C-19), 42.9 (C-14), 45.3 (C-9), 49.1 (C-17), 53.7 (C-18), 60.5 (C-5), 125.1 (C-12), 140.5 (C-13), 180.9 (C-28), 198.9 (C-24), 210.7 (C-3) (Figure 3).

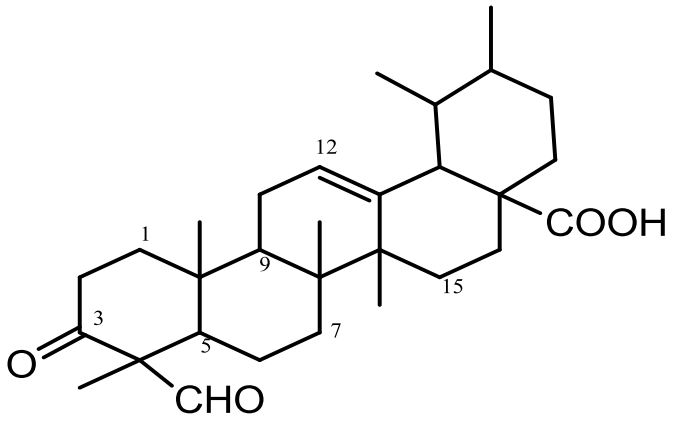

Fig. 3. Structure of Dioxo-urs-12-en-28-oic acid

Piceid

^{1}HNMR(400 MHz, DMSO): *δ* 9.50 (s, –OH), 7.28 (d, H-2', 6'), 7.11 (d, 16, H-b), 6.95 (d, 16.4 , H-a), 6.71 (d, H-3', 5'), 6.92 (s, H-2), 6.64 (s, H-6), 6.37 (s, H-4), 4.64–5.27 (sugar –OH), 3.79–4.27 (sugar C–H). ^{13}C NMR (100 MHz, DMSO) *δ*: 160.9 (C-3), 157.3 (C- 5), 156.7 (C-4'), 138.9 (C-1), 130.6 (C-1'), 129.0 (C-b), 126.9 (C-6' & 2'), 124.2 (C-a), 115.9 (C-3' & 5'), 119.3 (C-6), 110.3 (C-2), 105.8 (C-4), sugar C1-6: 104.8, 78.3, 76.1, 75.7, 68.6, 61.3 (Figure 4).

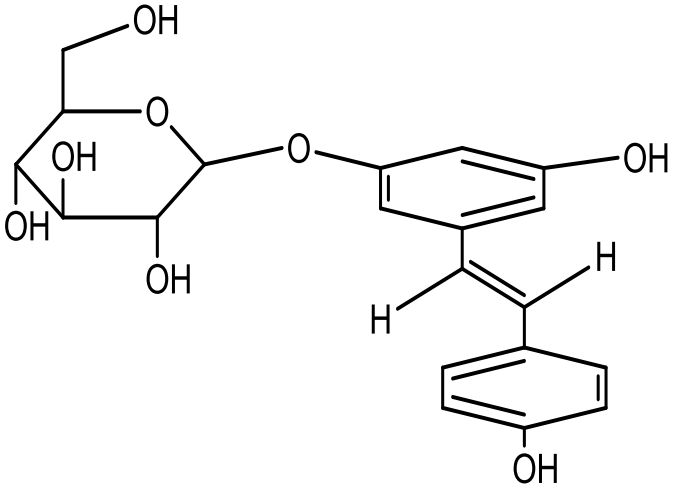

Fig. 4. Structure of Piceid

RESULTS AND DISCUSSION

Four compounds were successfully isolated from the leaves of *Cyphostemma digitatum.*

Longistylin A was obtained as a yellow colorless compound, it's phenolic compound having molecular

formula $C_{20}H_{22}O_2$ was clearly an asymmetrical bibenzyl based upon the presence of the characteristic isolated olefinic proton system resonating at δ 6.9 in the ^{1}HNMR spectrum ascribable to H- 7a and 8a, and showed one methylene proton at δ 3.2. One olefinic proton was observed at δ 5.73 ascribable to H- 2'. Exhibited a signal for aromatic protons at δ 7.72 (d, 7.5, H-2a & 6a), 7.45 (d, 7.4, H- 3a & 5a), 7.30 (dd, 7.3, 2, H-4a), 6.9 (d, 5.30, H- 7a & 8a), 6.43 (s, H-14a), 6.38 (s, H-10a) & a signal for methoxy protons (s, 3.86, 3H). ^{13}C NMR spectrum showed a methoxy group at δ 57.2. The measurements at δ 131.8, 127.1, 126.8, 123.5, were noticed for four olefinic carbons, corresponding to C-3, C-6, C-22 and C-23 respectively. Methyl carbons (C-4, C-5 and OCH_3) appeared in the most upfield regions of the NMR spectrum at δ 19.8, 25.6 and 57.2 correspondingly (Waterhouse and Lamuela-Raventos, 1995; Gollapudi *et al.,* 1989).

Lupeol was fractioned as a colorless gum, it has molecular formula $C_{30}H_{52}O_2$ as a pentacyclic triterpene. Mass spectrum of this compound demonstrated the molecular ion at m/z 426 resultant to the formula $C_{30}H_{50}O$, in agreement with other spectroscopic data. The ^{1}H NMR spectrum showed singlets for seven tertiary methyl, also one secondary hydroxyl group was present. Value δ 4.70, 4.61 showed olefinic protons for H-29a & 29b. ^{13}C NMR of compound demonstrated 30 signals for the terpenoid of lupine skeleton denoted by seven methyl groups. Hydroxyl group of C-3 appeared at δ 80.1, while the value at δ 151.1 and 109.5 appeared for alkene carbons (Chaturvedi *et al.,* 2008; Kuete *et al.,* 2007). The presence of lupeol in the *Cyphostemma digitatum* was not reported before the current study.

Dioxo-urs-12-en-28-oic acid was obtained as white powder. It's a triterpene having molecular formula $C_{30}H_{44}O_4$. ^{1}H NMR spectrum of this compound indicated the presence of six tertiary methyls, appearing at δ 0.87, 0.91, 1.05, 1.13, 1.21 & 1.31 for H-26, H- 29, H-30, H-25, H-23 and H-27, respectively. The signal of the vinylic proton H-12 appeared as a multiplet at δ 5.36 due to the coupling with methylene H-11. The signal of the aldehyde proton appeared at δ 9.68. ^{13}C NMR of the compound demonstrated 30 signals for the terpenoid skeleton denoted by six methyl groups. Carbonyl groups appeared at δ 198.9 and 180.9 for C-3 and C-28 respectively. While the double bond carbons appeared at δ 140.5 and 125.1 for carbons C-13 and C-12 respectively (Singh *et al.,* 1990; Yadav, 2003).

Piceid was obtained as pale amorphous powder, is a stilbenoid glucoside, have chemical formula $C_{20}H_{22}O_8$. The ^{1}H-NMR spectrum of Piceid showed two sets of signals. The former, was assigned to glycosyl protons, consistent with the ^{13}C-NMR spectrum, showed six signals characteristic of a D-glucose unit. While the double bond carbons appeared at δ 124.2, 129.0 for carbons C-a and C-b respectively (Chen *et. al.,* 2000). Khan et al. (2015) showed the presence of various compounds like alkaloids, flavonoids, saponins, coumarins, steroids, terpenoids and tannins in extracts of *C. digitatum*. Similarly another study has reported A previous study has reported the isolation of six triterpenoids derivatives of lupane-type structure from resin of *Dracaena cinnabari* Balf., endemic to Socotra island, Yemen (Masaoud *et al.,* 2015).

CONCLUSION

The isolation and identification of Longistylin A, LupeoL, Dioxo-urs-12-en-28-oic acid and Piceid, from the leaves of *Cyphostemma digitatum* was done and reported from this plant. The work was performed using various physical and spectral techniques.

ACKNOWLEDGEMENT

Thanks to Dr. Mohmmed Almaktari, Adel Abdu and Ramzy Alamery for the specimen identification and to Dr. Wan Yaccob Wan Ahmed from UKM for his help.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

Al-Duais, M., Müler, L., Böhm, V., Jetschke, G., 2009. Antioxidant capacity and total phenolics of *Cyphostemma digitatum* before and after processing - use of different assays. Eur. Food Res.Technol., 228 (5): 813-821.

Chaturvedi, P.K., Bhui, K., Shukla, Y., 2008. Lupeol Connotations for chemoprevention. Cancer Lett., 263(1): 1-13.

Chen L., F.Q. Yang, T.Y. Zhang, Y.S. Han, S.W. Chen, 2000. Fenxiceshi Xuebao. J. Instrum. Anal., 19: 60.

Khan, R., Saif, A.Q., Quradha, M.M., Ali, J., Rauf, A., 2015. Phytochemical analysis, antimicrobial, antioxidant and urease inhibitory potential of *Cyphostemma digitatum* Lam. Nat. Prod. Res., 29(5): 466-468.

Kuete, V., Komguem, J., Beng, V.P., Meli, A.L., Tangmouo, J.G., Etoa, F.X., Lontsi, D., 2007. Antimicrobial components of the methanolic extract from the stem bark of *Garcinia smeathmannii* Oliver (Clusiaceae). South Afr. J. Bot., 73(3): 347-354.

Al-Duais, M., 2009. Vegetation Ecology and Ethnobotany of Cyphostemma digitatum in the Western Highlands in Yemen. Thesis, Friedrich-Schiller-Universität Jena.

Gollapudi, S.R., Telikepalli, H., Keshavarz-Shokri, A., Velde, D.V., Mitscher, L.A., 1989. Glepidotin C: A minor antimicrobial bibenzyl from *Glycyrrhiza lepidota.* Phytochem., 28(12): 3556-3557.

Masaoud, M., Hussein, K., Ahmed,A.H., Al.Maqtari, M.A., 2015. Lupane-type triterpenoids derivatives from resin of the Socotra Dragon tree (*Dracaena cinnabari* Balf.,

agavaceae). World J. Pharm. Pharm. Sci., 4(4): 182-194.

Singh, S.K., Tripathi, V.J., Singh, R.H., 1990. 3β,24-Dihydroxyolean-12-en-28-olc acid-A pentacyclic triterpene acid from *Lantana indica*. Phytochem., 29: 3360-3362.

Yadav, S.B. Tripathi, V., 2003. A new triterpenoid from *Lantana camara.* Fitoterapia, 74: 320-321.

Waterhouse, A.L., Lamuela-Raventós. R.M., 1995. The Occurrence of Piceid (Polydatin), a Stilbene Glucoside, in Grape Berries. Phytochem., 37: 571-573.