Int J Adv Life Sci Res. Volume 3(3) 50- 54
https://doi.org/10.31632/ijalsr.20.v03i03.005

International Journal of Advancement in Life Sciences Research

Online ISSN: 2581-4877

journal homepage http://ijalsr.org

Original Article

Phytochemicals: Extraction, Isolation & Identification of Bioactive Compounds from *Aristolochia bracteolate*

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Abstract

Objective:To isolate, analyze and identify the phytochemical compositions from *Aristolochia bracteolate* leaves. Methods: Preliminary phytochemical analysis for four active constituents, extracted from powdered leaves with different solvents by using Soxhlet apparatus and carried out by different chromatographic techniques. The phytochemical characterizations were evaluated by NMR & MS. Results: This species exhibited & Identified four compounds representing 2.0 % of the total phytochemical composition. The Preliminary phytochemical analysis confirmed the presence of Hydroxystigmast-5-en-7-one, Oleanonic acid, 5,7,4'-trihydroxy-3,8-dimethoxyflavone & 7,9-dimethoxy-tariacuripyrone. All of them are report for first time from this Spp. Conclusions: From this study, can be concluded that report for the first time from *Aristolochia bracteolate* leaves, identified 4 pure chemical compounds, by analysis using various physical & spectral techniques.

Keywords: *Aristolochia bracteolate leaves; Hydroxystigmast-5-en-7-one, Oleanonic acid, 5,7,4'-trihydroxy-3,8-dimethoxyflavone, 7,9-dimethoxy-tariacuripyrone, NMR analysis.*

Introduction

From the ancient time, plants have been extensively used as curative agents for multiplicity ailments. Approximately (21,000) listed of plant species used around the world for medicinal purpose according to World Health Organization (Rastogi and Mehrotra 1990). In India about 2,500 plants species belongs to more than 1000 genera are being used in the indigenous system of medicine. India is tenth among the plants rich countries of world and fourth among the Asian countries (Manjunath, B. L., 1948). Many herbal remedies individually or in combination have been recommended in various medical treatments for the cure of different diseases (Tomar A. 2017a). *Aristolochia indica* a shrub or perennial herb, prostrate or twining belongs to the family *Aristolochiaceae* and well documented in Ayurveda and Unani system of medicine to treat different ailments (Shirwaikar & Somashekar, 2003). *Aristolochia* is an important genus of order Piperales from the sub-family Aristolochoideae belong to family Aristolochiaceae and represented by 500 species (Chauhan *et al.* 2009). *Aristolochiaceae* family has been recently the subject of more attention because of the suggestions that these basal angiosperms are phylogenetically close to the divergence of monocots from dicots. *Aristolochiaceae* was traditionally placed in subclass Magnoliidae and thought to be related to woody members of the subclass Annonales (Tomar, A. 2008). All members of Aristolochiaceae have

anatropous, bitegmic, crassinucellate, endostomic ovules with a few exceptions (Tomar, A. 2016). Aristolochia is the genus of shrubs, or rhizomatous perennial herbs, often twinning, mainly distributed throughout the tropics, but some species occur in warmer temperate regions also (Tomar, A. 2017b). The greatest diversity in Aristolochia species is found in Central and South America. Aristolochia known as contemporary medicine, is plant extracts have been used in therapies of arthritis, gout, rheumatism, and festering wounds (Tomar, A., 2017c). *Aristolochia bracteolata* Lam. is one of 277 medicinal

plants possessing abortificient, emmenagogue as well as ecobolic, carcinogenic and mutagenic properties of aristolochic acids (Kuo *et al.* 2012). *Aristolochia bracteolate* showed a definite positive effect on wound healing, with significant increase in the level of powerful antioxidant enzymes (Suliman *et al.* 2014). Its root and leaves were bitter and anthelmintic and are medicinally important. Almost every part of the plant has medicinal usage (Lopes *et al.* 2011). The whole plant was used as a purgative, antipyretic, and anti-inflammatory. It also possesses a potent anti-allergic activity (Tian-Shung *et al.* 2005).

Material and Methods

I. *General*

NMR experiments were performed on a Bruker Avance 2 (500 MHz) instrument at 500.13 MHz for 1H and 125.13 MHz for 13C. All spectra were recorded in CDCl3 using TMS as internal standard. UV spectra were obtained on a Hewlett Packard

8453 spectrophotometer and IR spectra were recorded on a Nicolet Magna 550 spectrophotometer. Vacuum flash chromatography was carried out either on silica gel (Aldrich Chemical Co.). All solvents were distilled prior to use. TLC plates were sprayed with 2% vanillin in concentrated H2SO4.

II. *Plant material*

Leaves of *Aristolochia bracteolate* were collected by student (YJU) at Ibb area, Yemen, in February 2015. Voucher specimens were identified by Dr. Adel Abdu (Universidad Sana'a, Yemen) and were deposited at the herbarium (YJU). Voucher numbers: AB2015.

III. *Extraction and Isolation of compounds*

Dried leaves of *Aristolochia bracteolate* were (1500 g) were diced and extracted

with aqueous ethanol (20%) for two days in Soxhlet apparatus . The combined extracts were concentrated under reduced pressure. The supernatant was evaporated

to dryness, giving a crude extract (11 g). The latter was subjected to vacuum flash chromatography on silica gel using a Petro / EtOAc (1:1) gradient ranging from 100% petro to 100% EtOAc, and ten fractions (AB1–AB14) were obtained. TLC analysis showed that fractions AB2, AB4, AB6,AB8, AB10 contained the compounds of concentration. Fraction AB2 (20 mg, eluted with EtOAc /petro 90:10) to yield Hydroxystigmast-5-en-7-one, AB4 (12 mg, eluted with EtOAc / petro 80:20) was purified by PTLC to yield Oleanonic acid, Fraction AB6 (16 mg, eluted with Petro/ EtOAc 60:40) known as 5,7,4'-trihydroxy-3,8-dimethoxyflavone, Separating the fraction AB8 on silica gel with Petro/EtOAc (70: 30) and purifying the products to yield 7,9-dimethoxy-tariacuripyrone.

Hydroxystigmast-5-en-7-one, Colorless granules; mp: 128-130 0C; 1H-NMR (500 MHz, CDCl3): δ 0.67 (3H, s, H-18), 1.20 (3H, s, H-19), 3.71 (1H, m, H-3), 5.67 (1H, d, H-6); 13C-NMR (100 MHz, CDCl3): δ 36.30 (C-1), 31.15 (C-2), 71.23 (C-3), 41.81 (C-4), 167.10 (C-5), 123.92 (C-6), 202.09 (C-7), 45.62 (C-8), 50.06 (C-9), 38.36 (C-10), 21.26 (C-11), 38.82 (C-12), 43.08 (C-13), 49.73 (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), 26.35 (C-23), 45.87 (C-24), 29.69 (C-25), 19.09 (C-26), 19.79 (C-27), 23.03 (C-28), 12.03 (C-29).

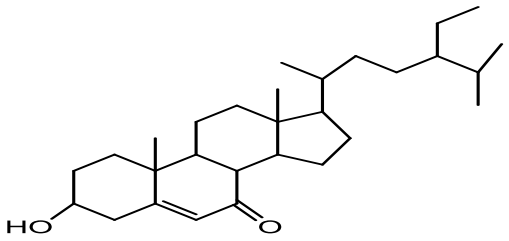

Figure (1) Hydroxystigmast-5-en-7-one

Oleanonic acid (2), Colorless needles; mp:279-280 °C; 1H NMR (500 MHz, CDCl3): δ 0.86, 0.94, 0.98, 0.98, 1.00, 1.13, 1.13 (each 3H, s, CH3 × 7), 2.62 (1H, dd, H-18), 5.41 (1H, m, H-12); 13C-NMR (100 MHz, CDCl3): δ 34.53 (C-1), 30.24 (C-2), 100.07 (C-3), 40.26 (C-4), 49.06 (C-5), 20.11 (C-6), 30.54 (C-7), 38.26 (C-8), 42.65 (C-9), 35.14 (C-10), 23.73 (C-11), 121.98 (C-12), 142.82 (C-13), 40.84 (C-14), 28.34 (C-15), 24.11 (C-16), 51.03 (C-17), 39.10 (C-18), 46.55 (C-19), 30.56 (C-20), 38.48 (C-21), 75.09 (C-22), 27.43 (C-23), 18.27 (C-24), 19.08 (C-25), 17.44 (C-26), 25.30 (C-27), 177.98 (C-28), 33.74 (C-29), 27.20 (C-30).

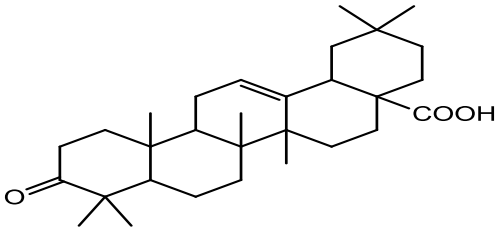

Figure (2) oleanonic acid

Trihydroxy-3,8-dimethoxyflavone (3) yellow needles. The 1H-NMR spectrum (400 MHz, CDCl3), δ 12.7 (OH, s, H-5), 7.94, (H-d, H-2l & 6l), 6.92, (H,d, H-3l & 5l), 6.54 (H,s,H-6), 3.78 (H,s, C-3 (-OCH3)), 3.69(H,s, C-8(OCH3)); l3C NMR (100 MHz, CDCl3): δ 178.41 (c-4), 161.09 (c-4l), 157.31 (c-7), 156.23 (c-2), 152.63 (c-9), 151.52 (c-5), 137.62 (c-3), 130.81 (c-8), 130.04 (c-2l), 129.81 (c-6l), 120.60 (c-1l), 115.34 (c-5l), 114.76 (c-3l), 104.52 (c-10), 94.27 (c-6), 60.25 (C-8-(OCH3)), 59.54 (C-3- (OCH3)).

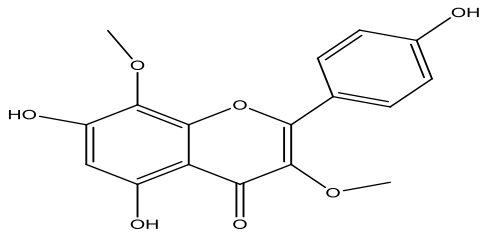

Figure 3, Trihydroxy-3,8-dimethoxyflavone

Dimethoxytariacuripyrone (4) Yellow powder, mp 224-226, 1H NMR (400 MHz, CDCl3), δ 4.05 (3H,s, 5-OMe), 4.02 (3H-s- 7-OMe), 4.00 (3H-s- 9-OMe), 6.54 (H-d- H-3), 6.82 (H-s- H-8), 7.50 (H-s- H-10), 8.94 (H-s- H-6), 7.05 (H-d- H-4); l3C NMR (100 MHz, CDCl3): δ 162.03 (C-9), 160.71 (C-2), 157.07 (C-7), 155.24 (C-5), 150.63 (C-10b), 139.36 (C-4), 126.07 (C-10a), 118.15 (C-3), 115. 32 (C-6a), 114.93 (C-6), 110.27 (C-4a), 100.04 (C-8), 99.05 (C-10), 59.12 (C- 5-OMe), 57.34 (C- 9-OMe), 55.67 (C- 7-OMe).

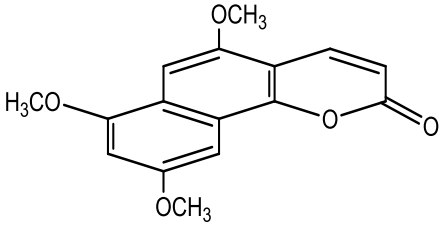

Figure 4, di-methoxy-tariacuripyrone.

Results and Discussion

The concentrated methanol extract of the leaves of *Aristolochia bracteolate* was repeatedly chromatographed over silica gel flash column chromatography, and compound 1-4 were eluted in the order of tier increasing polarity. The 1H and 13C NMR spectral data for these compounds revealed that 1 belong to steroids, compound 2, belong to the tri-terpene group, compound 3, belong to Flavonoids & compound 4, belong to coumarins. Were identified by using physical constants, spectral data and a direct comparison with an authentic sample.

Compound 1; was obtained as colorless granules; 1H-NMR (500 MHz, CDCl3) spectra showed the presence of six methyl's appeared at δ 0.63, 0.67, 0.76, 0.80, 0.87, 1.20.The proton of H-3 appeared as a multiplet at δ 3.71. It also showed olefinic protons at δ 5.67. 13C NMR showed twenty nine carbon signal including six methyles, nine CH2, eight CH & four quaternary carbons. The alkenes carbons appeared at δ 167.10, 123.92, also appeared signal for carbonyl at δ 202.09.

Compound 2; was obtained as colorless needles. The 1H NMR (500 MHz, CDCl3): displayed signals for olefinic proton signals at δH = 5.41 (1H, m, H-12); carboxylic acid proton at δH = 12.03 (1H,s) & displayed seven CH3 at δH = 0.86, 0.94, 0.98, 0.98, 1.00, 1.13, 1.13 (3H, s). Thirty carbon signals appeared in the 13C-NMR spectrum, including seven methyl carbon, one carboxylic carbons at δ C = 177.98, two olefinic carbons at δ C = 121.98, and 142.82, ten methylene carbons and ketone carbon at δ C = 100.07.

Compound 3; was obtained as yellow needles. The 1H-NMR spectrum displayed two CH3 carbons at δ H 3.78 and 3.69, show aromatic proton signals at δ H 7.94, 6.92 and 6.54 for ((H-2l & 6l), (H-3l & 5l) & (H-6)) respectively. While 13C-NMR spectrum suggesting a flavonol skeleton in compound. Spectra revealed the Signal at δ 178.41 ppm indicate carbonyl carbon (C-4) at normal low field position. Another signal at δ 161.09, 157.31, 151.52 Indicate C-4', C-7 & C-5 position of flavonol carbons. Signal at δ 156.23, 152.63, 137.62 & 130.81 attributed for C-2, C-9, C-3 & C-8 position of the carbon, Signal at δ 104.52 showed C-10 carbon position. Another signal at δ 94.27 indicate C-6 position of carbon, methyl group appeared signals at δ 60.25 & 59.54 at position C-8 & C-3.

Compound 4; was obtained as Yellow powder. The 1H-NMR spectrum displayed two proton doublets at δH 6.54 & 7.05, characteristic of the H-3 and H-4 of the isolated compound in the coumarin rings. The presence of further three proton singlet at δH 6.82, 7.50 & 8.94 indicated the presence of H-8, H-10 and H-6. Also displayed a proton singlet at δ H 4.00, 4.02 & 4.05 (s) characteristic of a methoxy group (5-OMe), (7-OMe) & (9-OMe). The 13C-NMR spectrum which displayed a total of 16 carbon resonances. It showed thirteen carbon resonances for the furanocoumarin nucleus, and three additional signals arising from carbons at the side chain that accounted for: 3 methoxy groups (δC 59.12, 57.34 & 55.67.

Conclusion

Four our compounds (Hydroxystigmast-5-en-7-one, Oleanonic acid, 5,7,4'-trihydroxy-3,8-dimethoxyflavone & 7,9-dimethoxy-tariacuripyrone) were isolated from the aerial parts and the leaves of *Aristolochia bracteolate*. Among them, Hydroxystigmast-5-en-7-one & Oleanonic acid are reported for the first time from the spp. The isolated compounds were identified by measuring its melting point, chromatography methods and NMR spectroscopy.

Acknowledgments:

The author is thankful for the University Management for giving permission and necessary support to carry over the work

Conflicts of Interest:

The authors declare that the research was conducted in the absence of any commercial or economic associations that could be construed as a potential conflict of interest

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