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RESEARCH ARTICLE

TWO BIOACTIVE CONSTITUENTS FROM *ARGEMONE MEXICANA*

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Abstract

The chemical investigation of the methanol extract of aerial parts of *Argemone mexicana* belonging to the family Papaveraceae led to the isolation of two bioactive phytoconstituents namely stigmasterol and quercetin. The isolated compounds were characterized using various spectroscopic data as well as chemical studies. Out of the two compounds isolated, stigmasterol has been reported for the first time from the plant.
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Keywords: *Argemone mexicana*, Papaveraceae, Methanol extract, Aerial parts, Bioactive constituents.

Introduction

Argemone mexicana L., known as Ghamoya (family: Papaveraceae) is an exotic weed indigenous in South America and has widespread distribution in many tropical and sub-tropical countries including West Africa (Ibrahim and Ibrahim, 2009). This plant is common everywhere by roadsides and fields in India also (Bhalke and Gosavi, 2009). The plant is an erect prickly annual herb of about 1 m high; leaves are usually 5 to 11 cm long, and more or less blotched with green and white, glaucous broad at the base, half-clasping the stem prominently sinuate-lobed, and spiny (Chopra *et. al*., 1956). The flowers become 4 to 5 cm in diameter, and are terminal, yellow, and scentless. The capsule is spiny, obovate or elliptic-oblong, and about 3 cm in length. The seeds are spherical, shining, black and pitted. *A. mexicana* is considered as an important medicinal plant in India; the yellow juice, which exudes when the plant is injured, has long been used in India as traditional medicine for dropsy, jaundice, ophthalmia, scabies and cutaneous affections (Chopra *et. al*., 1956; Ambasta, 1986; Sharma *et. al*., 2012). Different parts of this plant are used in chronic skin diseases, and also as emetic, expectorant, demulcent and diuretic; the seeds and seed oil are employed as a remedy for dysentery, ulcers, asthma and other intestinal affections (Chopra *et. al*., 1956; Ambasta, 1986; Bose *et. al*., 1963; Prajapati *et. al*., 2003; Savithramma *et. al*., 2007). Leaves and seeds are also reported to find application in maintaining normal blood circulation and cholesterol level in human body (Albuquerque *et. al*., 2007); these plant parts possess anti-venom property as well (Makhija and Khamar, 2010; Minu et. al., 2012). Flowers are found to be expectorant and have been used in the treatment of coughs (Brahmachari *et. al*., 2010). In Brazil, the plant is commonly known as 'cardo-santo' and used traditionally in the treatment of a number of diseases (Agra *et. al*., 2007 and 2008; Bieski *et al*., 2012). Seeds of the plant are used as purgative, laxative and digestive while its latex is used against conjunctivitis (Agra *et. al*., 2008). Besides, its infusion finds application against hypertension in Brazil (Bieski *et al*., 2012). Previous phytochemical study on this plant reveals the presence of alkaloids, terpenoids, steroids, flavonoids, phenolics, long-chain compounds, sugars, tannins etc. (Brahmchari *et. al*., 2013). Herein, we report the isolation and characterization

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of two known bioactive constituents—stigmasterol and quercetin from the aerial parts of the plant.

Materials and Methods

Phytochemical Investigation

General experimental procedures

All the melting points were recorded on Model No. Chemiline-715 melting point apparatus and were uncorrected. IR measurements were obtained on Perkin-Elmer (FT-IR) infrared spectrophotometer. TMS has been used as internal standard in recording ^{1}H-NMR spectra (Bruker DRX300; $CDCl_3$, 400 MHz), ^{13}C-NMR Spectrum was performed on 100 MHz instrument (Bruker DRX 300) using TMS as internal standard and EIMS Spectrum was carried out on JEOL-JMS 600 (70 eV). TLC was carried out using Silica-gel 60/ UV254 using precoated plates. Silica-gel ('Merck' 60-120 mesh) was used for Column Chromatography.

Plant material

Aerial parts of *A. mexicana* were collected from Santiniketan, Birbhum District, West Bengal, India during July, 2013. It was authenticated by Dr. H.R. Choudhury, Department of Botany, Visva-Bharati, Santiniketan, West Bengal, India. A voucher specimen of the plant has been kept in the Department of Chemistry, Kulti College, Kulti, Burdwan.

Preparation of the methanol extract

The aerial parts of *A. mexicana* were collected and dried in shade. The dried bark was powdered (1kg) and exhaustively extracted by Soxhlet apparatus with methanol for 56 h. Then the alcohol layer was decanted off. The solvent of the extract was distilled off by using rotary evaporator and the brown syrupy material thus obtained was allowed to evaporate to dryness and a brown mass (about 15.5 g) was obtained. The preliminary phytochemical studies were performed for testing the different phytoconstituents present in the methanol extract. The chemical tests revealed the presence of steroids and flavonoids.

Isolation of compounds from methanol soluble fraction

The methanol soluble fraction (12.5 g) was dissolved in minimum volume of methanol and adsorbed onto silica gel (60-120 mesh, 36 g). After evaporation of the solvent, the resulting mass was loaded on column packed with about 190 g of Silica-gel prepared in Petroleum ether (60-80 ^{0}C). The column was then eluted with different solvents with increasing polarity starting from *n*-hexane (100%) and ending with methanol (100%). The elutions were monitored by TLC (Silica gel-G; visualization by UV 254 nm, 366 nm and Vanillin-Sulphuric acid spraying reagent heated at 110 ^{0}C).

Elution carried out with Chloroform: ethylacetate in 3:2 ratio resulted a single white amorphous solid compound and designated as **Compound 1** (94 mg).

Elution carried out with Chloroform: ethylacetate in 1:10 ratio resulted a pale yellow solid compound and designated as **Compound 2** (84 mg).

During elution with different solvents, compounds with very low quantity or mixtures of many compounds were collected occasionally but unable to process further analysis due to lack of infrastructures (unavailability of GC-Mass etc.) in our and nearby institutions.

Characterization

Stigmasterol (1): White amorphous solid; mp 169-170 ^{0}C. The compound **1** gave a red colour in Salkowski's test and a green colour in Libermann-Burchard's test specific for steroids. IR (KBr) ν_{max} 3375.4 cm^{-1} (broad, OH), 2937.8 cm^{-1} (C-H stretching in CH_3), 2837.6 cm^{-1} (C-H stretching in CH2), 1657.1 cm^{-1} (C=C stretching), 1454.5 cm^{-1} (C-H deformation in gem dimethyl groups), 1026.14 cm^{-1} (C-O stretching in secondary alcohol); ^{1}H NMR ($CDCl_3$): δ 0.98 (3H, s, H-21), 0.87 to δ 0.91 (9H, m, H-26, H-27, H-29), 0.85 (3H, s, H-18), 0.854 (3H, s, H-19), 1.28 to 1.65 (18H, m, 9 x CH_2 and 7H, methine protons), 2.79 (1H, s, H-3), 2.01 to δ 2.39 (1H, m, OH), 5.37 (1H, s, vinylic protons), 5.32 to 5.64 (2H, m, H-22 and H-23); ^{13}C NMR ($CDCl_3$): δ 140.9 (C-5), 138.5 (C-22), 129.5 (C-23), 121.9 (C-6), 72.0 (C-3), 57.0 (C-14), 56.1 (C-17), 51.4 (C-24), 50.3 (C-9), 46.0 (C-25), 42.4 (C-13), 40.7 (C-20), 39.7 (C-12), 37.5 (C-4), 37.4 (C-1), 36.7 (C-10), 32.1 (C-8), 31.9 (C-7), 29.2 (C-16), 28.4 (C-2), 25.6 (C-28), 24.5 (C-15), 21.4 (C-21), 21.3 (C-11), 20.0 (C-27), 19.6 (C-26), 19.1 (C-19), 12.2 (C-29), 12.1 (C-18); EIMS (70 ev): *m/z* 412 $[M]^{+}$, 351, 314, 300, 271, 229, 213, 55. All data are identical with that of stigmasterol [Jamal *et. al*., 2008; Sarwar Alam *et.*

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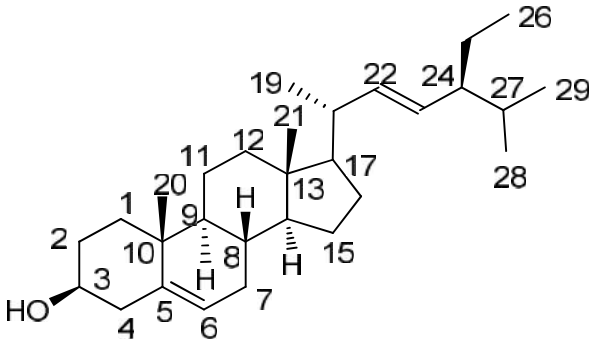
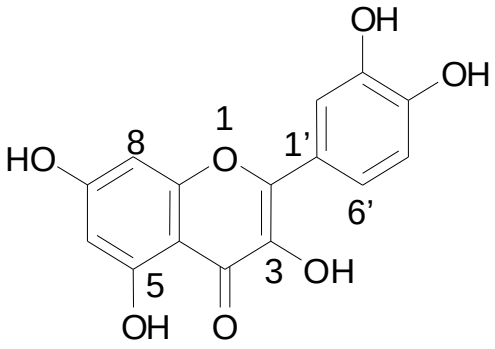
al., 1996; Singh *et. al*., 1978; Chawla *et. al*., 1974; Jash *et. al*., 2013).



Stigmasterol

Quercetin (2): Yellow amorphous powder, mp 311-313^{0}C. The compound **2** responded Shinoda test for flavonoids. UV (MeOH): λ_{max} 259, 368 nm; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 6.17 (1H, *d*, *J* = 2.0 Hz, H-6), 6.40 (1H, *d*, *J* = 2.0 Hz, H-8), 6.88 (1H, *d*, *J* = 8.0 Hz, H-5'), 7.61 (1H, *dd*, *J* = 2.0, 7.5 Hz, H-6'), 7.72 (1H, *d*, *J* = 2.0 Hz, H-2'); ^{13}C NMR (MeOH, 100 MHz): δ 147.7 (C-2), 137.2 (C-3), 177.5 (C-4), 162.4 (C-5), 99.3 (C-6), 165.6 (C-7), 94.5 (C-8), 158.3 (C-9), 104.5 (C-10), 124.2 (C-1'), 116.1 (C-2'), 146.3 (C-3'), 148.8 (C-4'), 116.3 (C-5'), 121.6 (C-6'). All data are identical with that of quercetin (Agrawal, 1989; Harborne, 1994; Liu *et. al*., 2003).



Quercetin

Results and Discussion

Chromatographic separation of the methanol extract of aerial parts of *A. mexicana* led to the isolation of two phytoconstituents belonging to the category of steroids and flavonoids. They were characterized as stigmasterol and quercetin using spectroscopic techniques like IR, ^{1}H NMR, ^{13}C NMR and EIMS as well as chemical studies. Both these phytochemicals are well established as significant bioactive compounds (Adebamowo *et. al*., 2005; Bosetti *et. al*., 2005; Cruz-Correa *et. al*., 2006; Gabay *et. al*., 2010; Panda *et. al*., 2009; Yinusa *et al*., 2014]. Stigmasterol has been isolated for the first time from this plant.

Conclusion

The phytochemical investigation of the methanol extract of the aerial parts of *A. mexicana* belonging to the family Papaveraceae was successfully carried out. The chemical constituents isolated from this extract must account for the biological activities exhibited by the crude methanol extract of the plant. Therefore, it is now turn of the pharmacologists/biologists to explore the plant more systematically by carrying out individual bioactivity of the isolated chemical constituents. Therefore, the present work will boost the scientific communities to do more work on this important medicinal plant in near future.

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