Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 12, 2015

Original Article

ISOLATION AND CHARACTERIZATION OF STIGMASTEROL FROM CHLOROFORM FRACTION OF AERIAL PART OF *ARGEMONE MEXICANA* L

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Received: 30 Sep 2015 Revised and Accepted: 27 Oct 2015

ABSTRACT

Objective: Phytosterols are group of steroidal alcohol play important roles in structural component in the cell membrane and play a role in membrane stability. There are almost 22 different sterols are found yet and the major phytosterols include ß-sitosterol, campesterol and stigmasterol. The objective of this study was to isolate and characterize the bioactive principles from the chloroform fraction of *Argemone Mexicana* L.

Methods: The isolation was done using column chromatography using gradient elution with different mobile phases. The isolated compound was subjected to spectral analysis. Structure elucidation was carried out on basis of spectral analysis.

Results: The chemical investigation of the chloroform fraction of aerial parts of belonging to the family Papaveraceae led to the isolation of stigmasterol. The isolated compounds were characterized using various spectroscopic data as well as chemical studies.

Conclusion: From the spectral characteristics, the isolated compound from the chloroform fraction of aerial parts was confirmed to be stigmasterol. This is the first ever report of these stigmasterol compound from the chloroform fraction of aerial parts of *Argemone mexicana*.

Keywords: Phytosterols, *Argemone mexicana,* Stigmasterol, Isolation, Papaveraceae.

INTRODUCTION

Plants have shaped the basis of sophisticated traditional medicine systems that have been used for thousands of years in countries, such as China [1] and India [2]. The use of plants in the traditional remedy of many other cultures has been widely documented. These plant-based systems continue to play an significant role in health care and it has been projected by the World Health Organization that around 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care, although plant products also play an main role in the health care systems of the remaining 20% of the population mostly residing in developed countries [3].

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 [4]. This plant is common every whereby roadsides and fields in India also [5]. 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 [6]. 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, opthalmia, scabies and cutaneous affections [6-8]. 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 [6, 7, 9-11]. Leaves and seeds are also reported to find application in maintaining normal blood circulation and cholesterol level in human body [12].

The plant *Argemone mexicana* is the source of a different kind of chemical constituents having mostly abundant alkaloids as berberine [13], protopine, nor-sanguinarine [14], sanguinarine [15], Angeline [15], chelerythrine [16] etc. Other active constituents which are found are terpenoids {trans-phytol [16], β-amyrin [17]}, steroids {β-sitostero [18]}, long-chain alcohols {myristic acid, palmitic acid, stearic acid, arachidic acid, oleic acid and linoleic acid [19], argemonic acid [20]}, fatty acids from seed oil {9-and 11-oxo-octacosanoic and 11-oxotriacontanoic acids}, amino acids {cysteine and phenylalanine}[20], flavonoids {luteolin, eriodyctiol [21], isorhamnetin-3-O-β-Dglucopyanoside [16-22], iso rhamnetin [22, 23] isorhamnetin-7-O-β-Ddiglucopyanoside [23], isorhamnetin-3,7-O-β-Ddiglucopyranoside, quercetin, rutin, mexitin [24, 25]}, aromatic acids {5,7-dihydroxy chromone-7-neohesperidoside [26], tannic acid, caffeic acid, ferulic acid [27], vanillic acid [22]}, miscellaneous compounds {α-tocopherol, adenosine, adenine}[16].

In the present study, first time we have isolated and characterized stigmasterol, a phytosterol, from the chloroform fraction of aerial parts of *Argemone mexicana* L. Stigmasterol is reported to exhibit a spectrum of pharmacological activities against various disease conditions. These include conditions such as inflammation, arthritis, diabetes, cardiovascular ailments, renal disorder, hepatic toxicity, microbial infections and cancer.

MATERIALS AND METHODS

Collection and authentification of plant

The plant material used in this study was aerial parts of *A. mexicana,* collected from Kasrawad dist. Khargone M. P., India, during spring (mid-March to mid-April) and was authenticated by the Former Taxonomist Dr. S. K. Mahajan, department of Botany, Government P G College Khargone M. P. The plant materials were initially rinsed with distilled water and dried on paper towel in laboratory at (37±1) °C for 24 h.

Extraction and fractionation

The coarse powder of *A. mexicana* was submerged in alcohol and water (50:50) and allowed to stand for continuous hot extraction. After extraction the solvents were allowed to evaporate using rotary evaporator at temperature 40-45 °C. Thus the highly concentrated crude hydroalcoholic extract were obtained. They were then fractionated using Petroleum ether, Chloroform and water. The chloroform fractions obtained from *A. mexicana* was then stored in a refrigerator at 4 °C for further use for phytochemical investigation.

Isolation of compounds from chloroform fraction of *A. mexicana*

The dried chloroform fraction of *A. mexicana* (20 gm) was mixed with 80 gm silica gel (60-120 mesh) to make the material to get

adsorbed in the silica gel. The column was eluted with solvent increasingly initial from 100 % n-hexane, then increasing order of ethyl acetate in n-hexane (0, to 100% ethyl acetate in n-hexane) and total 108 fractions were collected. After evaporating the solvents on water bath all the collected fractions were subjected to TLC analysis. On the basis of Rf values, same fractions were pooled together. The pools which gave single spot in iodine exposure were 8-19, 28-35, and 74-86. On the basis of high quantity, 74-86 pool of fractions was taken for purification.

Purification of the isolated compound from *A. mexicana*

Fractions from column chromatography were subjected to preparative TLC as required to obtain pure compounds. Mixed fractions of 74-86, after evaporating solvent, was subjected for thin layer chromatographic study using various solvent systems. Among them the Toluene: Acetone (8:2) gave good resolution this solvent system was further selected for preparative TLC. The concentrated pool was dissolved in chloroform and the sample was spotted in the preparative TLC plates. The sample applied plates were kept in completely saturated chamber of selected mobile system. After development of chromatogram, the plates were put in iodine vapour and the band (Rf =0.64) was identified and scrapped out from the plates. The scrapped material was then dissolved in pet ether and filtered through whatman filter paper. The filtrate was concentrated and the isolated product was obtained as white solid crystals named as compound AM (64 mg).

Test for steroid

Salkowski reaction

A few crystals of compounds AM were dissolved in chloroform and a few drops of concentrated sulphuric acid were added to the solution, compounds AM formed a reddish color in the upper chloroform layer [28] indicating presence of steroids.

Liebermann-Burchard reaction

A few crystals of compounds AM were dissolved in chloroform and few drops of concentrated sulfuric acid were added to it followed by the addition of 2-3 drops of acetic anhydride. In this case compounds AM turned to violet blue and finally formed green color which indicates the presence of steroids [28].

Spectroscopic characterization

UV spectra of the isolated compounds were recorded in methanol over a scanning range of 200-400 nm and λmax of compounds were determined. Spectra were recorded with a Shimadzu 1700 double beam-UV-VIS spectrophotometer. EIMS (electron impact mass spectrum) in positive mode were recorded on Waters Micromass Q-Tof Micro mass spectrometer instrument at SAIF, Chandigarh. The isolate was mixed with 200 mg KBr (FT-IR grade) and pressed into a pellet. The sample pellet was placed into the sample holder and FT-IR spectra were recorded in the range 375-7500 cm-1 in FT-IR spectroscopy (Model RZX (Perkin Elmer) at SAIF, Chandigarh. 1H and ^{13}C-NMR spectra were recorded on a FT-NMR Cryomagnet Spectrometer 400 MHz (Bruker) using TMS as an internal standard at SAIF, Chandigarh, India. The solvents used were methanol and DMSO. Chemical shifts ware shown in δ values (ppm) with TMS as an internal reference. For column chromatography silica gel 60 (70-230 mesh, Merck, Darmstadt, Germany) was used. Solvents for chromatography were distilled before use. Thin layer chromatography (TLC) was performed using TLC plates (Silica Gel G-60).

RESULTS

The melting point of compound AM was 169 °C; the UV λ_{max} value of compound AM was 257 nm. Mass spectrum of isolated compound AM showed parent molecular ion [M$^+$] peak at mlz 412 which corresponds to the molecular formula $C_{29}H_{48}O$ (fig. 1).

In the IR spectrum of isolated compound a very intensely broad peak at 3428 cm^{-1} and moderately intense peak at 1192 and 699 cm^{-1}were observed for the O-H bond vibrations of hydroxyl group. In the ^{1}H-NMR spectrum of isolated compound, H-3 proton appeared as a triplet of a double doublet (tdd) at δ3.20 and, H-6 olefinic proton showed a multiplet at δ5.24. Two olefenic protons appeared downfield at δ 4.57 m and δ 4.14 m. Six methyl protons also appeared at δ 1.23, δ 1.19, δ 1.06, δ 1.00, δ 0.98 and δ 0.91 (3H each, s, CH_3) (table 1).

The ^{13}C-NMR has shown recognizable signals at 140.8 and 121 ppm, which corresponds to double bond at C-22 and C-6 double bonds respectively as well as it also represent signals at 130.1 and 129.1 ppm, which shows one more double bond in between C-5 and C-23. The δ value at 71.6 ppm is due to C-2 β-hydroxyl group. The signal at δ 31.7 and δ 12.8 ppm corresponds to angular carbon atom at C-25 and C-27 respectively (table 1). From the above observations, isolated compound was found to be stigmasterol.

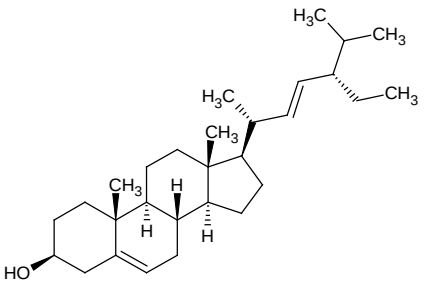

Fig. 1: Structure of stigmasterol isolated from chloroform fraction of *A. Mexicana.*

Table 1: Spectroscopic data of isolated compound am from chloroform fraction of *A. mexicana*

Spectroscopic techniques	Data
UV λ max	257 nm
IR (ranges in cm^{-1}) ($CHCl_3$)	3428 (O-H stretching), 2937(C-H stretching), 2852(C-H stretching), 1642 (C=C stretching), 1465 (C-H bend.), 1460 (C-H bend.), 1192 (O-H bend.), 1053 (C-C str.), 739 (CH_2 rocking), 699 (O-H bend.).
^{1}HNMR (DMSO)	δ 5.24 (m, 1H, H-6), δ 4.57 (s, 1H), δ 4.14 (s, 1H), 3.20 (tdd,OH,H-3), δ 1.23 (s, 3H), δ 1.19 (s, 3H), δ 1.06 (s, 3H), δ 0.98 (s, 3H), δ 0.91 (s, 3H).
^{13}CNMR (DMSO)	δ 140.8 (C-22), δ 130.1 (C-5), δ 129.1 (C-23), δ 121(C-6), δ 71.6 (C-3), δ 56.1 (C-4), δ 55.1 (C-5), δ 52.2(C-24), δ 0.10 (C-17), δ 43.8 (C-9), δ 41.2 (C-13), δ 39.4 (C-10), δ 37.7 (C-10), δ 33.4 (C-20), δ 31.7 (C-25), δ 29.1 (C-21), δ 28.1 (C-23), δ 25.1 (C-12), δ 21.8 (C-11, C-25, C-26), δ 15.1 (C-29), δ 12.8(C-27).
EIMS (70 ev): m/z with % abundance	412 [M+, $C_{29}H_{48}O$] 355(101), 311 (49), 301 (49), 279 (71), 219 (60), 200 (65), 175 (95)

DISCUSSION

The isolated phytochemical was found as a white amorphous solid compound with melting point of 169-170 °C. The UV λ_{max} value was 257 nm. In IR spectrum of AM, a very intensely broad peak at 3428 cm^{-1} and moderately intense peak at 1192 and 699 cm^{-1}were observed for the O-H bond vibrations of hydroxyl group. The out of plane C-H vibrations of the unsaturated part was observed at 881 cm^{-1}. The corresponding C=C vibrations was shown around 1642 cm^{-1} as weakly intense peak. The stretching and bending vibrations of methyl part were noticed by the intense peak 2937 cm^{-1}and medium intensity peak at 1465 cm^{-1}. The vibration of the methylene part was shown by the peak at 2852 cm^{-1} and the medium peak at 1460 cm^{-1}. The moderately intense peak at 739 cm^{-1}was attributed to the rocking movement of methylene part. The corresponding C-C vibration was shown as weak intense peak at 1053 cm^{-1}.

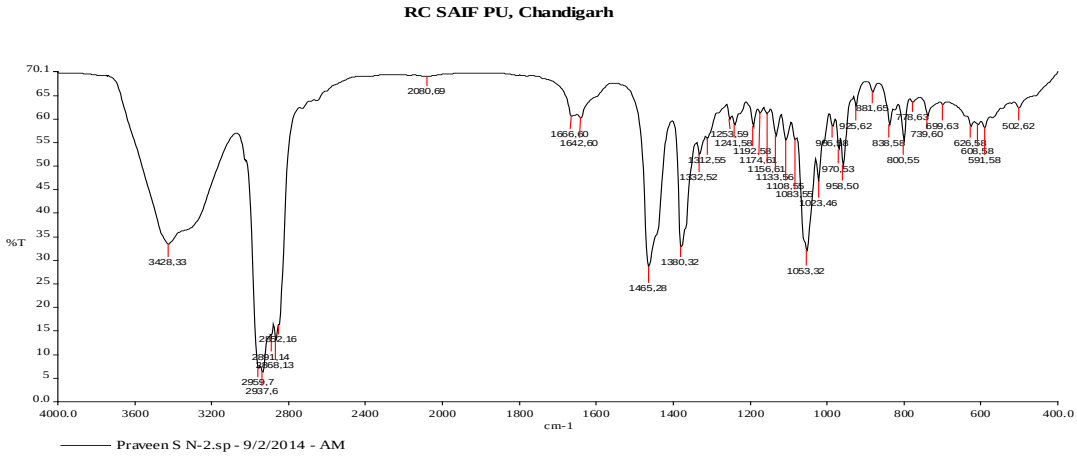

Fig. 2: IR spectra of the isolated lead compound

In ^{1}H-NMR spectrum of AM, H-3 proton appeared as a triplet of a double doublet (tdd) at δ 3.20 (J = 4.5 and 1.1 MHZ) and H-6 olefinic proton showed a multiplet at *S* 5.24. Two olefenic protons appeared downfield at δ 4.57 (m) and δ 4.14 (m) which were identical with the chemical shift of H-22 and H-23, respectively of stigmasterol [29]. Six methyl protons also appeared at δ 1.23, δ 1.19, δ 1.06, δ 1.00, δ 0.98 and δ 0.91 (3H each, s, CH_3).

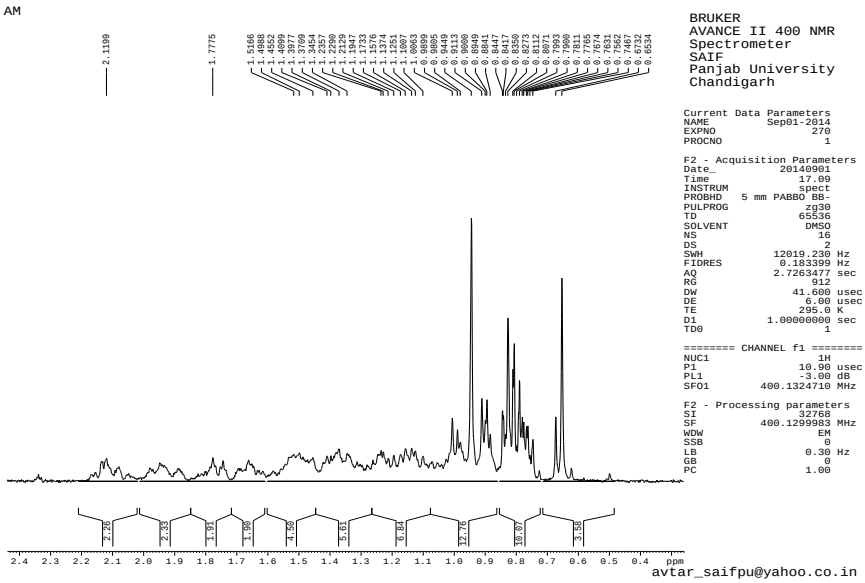

Fig. 3: ^{1}H-NMR spectra of the isolated lead compound

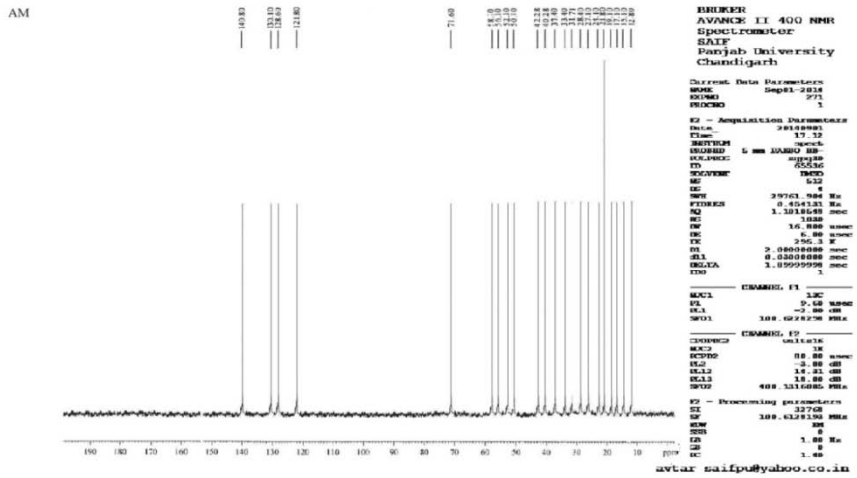

Fig. 4: ^{13}C-NMR spectra of the isolated lead compound

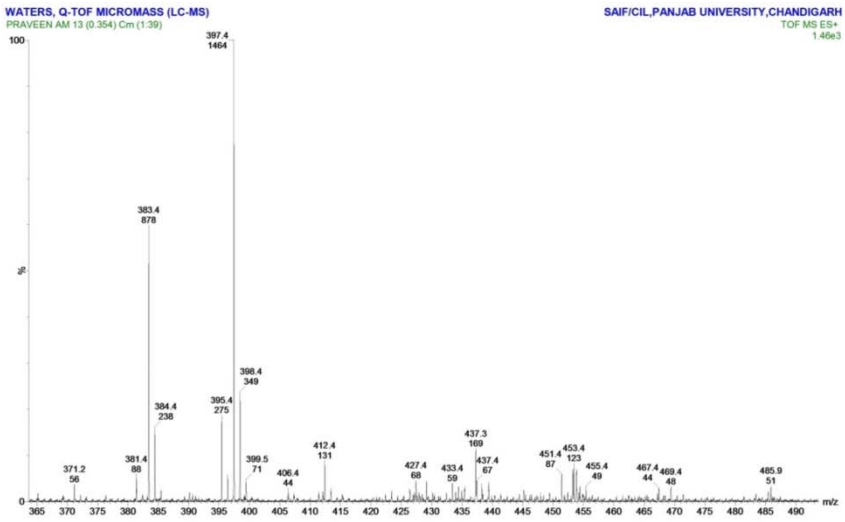

Fig. 5: Mass spectra of the isolated lead compound

The ^{13}C-NMR has shown recognizable signals at 140.8 and 121. ppm, which corresponds to double bond at C-22 and C-6 double bonds respectively as well as it also represent signals at 130.1 and 129.1 ppm, which shows one more double bond in between C-5 and C-23. The δ value at 71.6 ppm is due to C-2 β-hydroxyl group. The signal at δ 31.7 and δ 12.8 ppm corresponds to angular carbon atom at C-25 and C-27 respectively. Mass spectrum of compound AM showed parent molecular ion [M^+] peak at mlz 412 which corresponds to the molecular formula $C_{29}H_{48}O$. This assignment is overall in good agreement for the structure of Stigmasterol as described by [30-33].

CONCLUSION

The phytochemical examination of the chloroform fraction of the aerial parts of *A. mexicana* belonging to the family Papaveraceae was effectively carried out. From these physical, chemical and spectral evidences compound AM was confirmed as Stigmasterol (fig. 1). The stigmasterol isolated from this fraction must account for the biological activities exhibited by the chloroform fraction of the plant. Consequently, it is now turn of the pharmacologists/biologists to investigate the plant more thoroughly by carrying out individual bioactivity of the stigmasterol. So, the present work will enhance the scientific communities to do more work on this important medicinal plant in near future.

ACKNOWLEDGEMENT

The authors are grateful to SPS, SOA University, Bhubaneswar for providing necessary facilities to carry out the research work in the faculty of pharmacy, SOA University. Thanks are also to sophisticated analytical instrumentation facility (SAIF), Chandigarh for providing spectroscopic analytical data.

CONFLICT OF INTERESTS

Declared None

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