Objective: To investigate the photochemical constituents present in methanol extract of *Martynia annua* seeds using Gas Chromatography-Mass Spectroscopy (GC-MS), High-Performance Thin-Layer Chromatography (HPTLC) analysis and study antioxidant activity.

Methods: Methanol extract of *Martynia annua* seeds were subjected to GC-MS and HPTLC analysis. HPTLC analysis was carried out using GAMAG chromatography (HPTLC) analysis and study antioxidant activity. GC-MS analysis provided 17 peaks indicating the presence of seventeen different phytochemicals in methanol extract of *Martynia annua* seeds. HPTLC fingerprint showed 6 peaks at both size 2 µl and 5 µl at 254 nm whereas 4 peaks, 9 peaks were detected at 366 nm for 2 µl and 5 µl respectively. After derivatization with 10 % methanolic sulphuric acid, 8 peaks, 11 peaks were detected for 2 µl and 5 µl respectively when the derivatized plate was scanned at 540 nm. DPPH free radical scavenging result showed EC₅₀ value of 44.1±1.1 µg/ml.

Conclusion: The GC-MS analysis showed the presence of fatty acids, ester, aldehydes and ketones whereas in HPTLC different peaks at different UV-lights before and after derivatization were observed. Maximum percentage inhibition using DPPH assay was found 74 at concentration of 50 µg/ml.

Keywords: *Martynia annua*, GC-MS, HPTLC fingerprint, Antioxidant activity

MATERIALS AND METHODS

Chemicals and regents

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) was purchased from sigma-aldrich (USA). Ascorbic acid and butylated hydroxytoluene (BHT) were purchased from SD Fine Chemicals Pvt Ltd. All other reagents were analytical grade.

Plant material collection

*Martynia annua* seeds were purchased from local vender-Aurangabad. It was authentication by Dr. Narayan Pandara Department of botany Dr. Babasaheb Ambedkar Marathwada University-Aurangabad. Plant seeds were washed, dried in shadow at room temperature for more than 20 d, ground into powder form and kept in an airtight container in dark place till the time of use.

Preparation of extract

50 mg of dried *Martynia annua* seeds powder was extracted with 95% methanol (500 ml) by using Soxhlet apparatus for 6 h. After extraction, the solvent was removed using rotary vacuum evaporator; free solvent extract was kept in amber bottle in refrigerator.

Gas chromatography-mass spectroscopy (GC-MS)

GS-MS analysis of methanol extract of *Martynia annua* seeds was carried out in Indian Institute of Technology (IIT)–Madras. GC-MS analysis was done by using JEOL GC MATE II (GC model) equipped with HP 5 MS column. High purity helium as carrier gas at a constant flow rate of 1 ml/min was used for GC separation. Injector temperature was set at 220 °C and Oven temperature was set as 50 °C raised to 250 °C at 10 °C/min. Total GC running time was 30 min.

High sensitive quadruple double focusing mass analyzer was used and equipped with photon multiplier tube as the detector; mass range of 50 to 600 amu; and ionization voltage (Electron impact ionization) 70 eV was used.
High performance thin-layer chromatography (HPTLC)

HPTLC study was performed by following Reich and Schibli guidelines [17].

Sample application

The plant extract was dissolved in chromatographic grade methanol, two and five µl of extract solution individually was loaded as 8 mm band length in the plate format 200 ml ×100 mm Merck, TLC plate’s silica gel 60 F254 using LINOMAT 5 applicator attached to CAMAG HPTLC system which was programmed through winCAT software, version 2.5.1805.5.1

Spot development

After the application of sample, the chromatogram was developed in twin Trough Chamber (TTC) 20 ×10 cm which saturated with mobile phase Toluene: Chloroform: Ethanol (4:4:1 V/V/V), saturated time 20 min. After development, the plate was dried at room temperature for 5 min.

Photo-documentation

The plate In Photo-Documentation under GLP (Camag Visualiser), images were captured at white light, UV 254 nm and UV 366 nm

Scanning

The plate was fixed in CAMAG TLC Scanner 4 and scanning was done at 540 nm (Absorption, Lamp: Tungsten) for 5 min. The developed plate was immersed in immersion device which contains 10% methanolic sulphuric acid with dipping speed 5 mm/sec., the derivatized plate was heated at 100 °C for 3 min, the plate was photo-documented in white light and 366 nm. The scanning for a derivatized plate was done at 540 nm (Absorption, Lamp: Tungsten).

DPPH free radical scavenging assay

The antioxidant activity of the extract was determined using 1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH). DPPH scavenging activity was measured by the spectrophotometric method with minor modification (Sreejayan and Rao, 1996) [18]. 0.05 ml of the extract dissolved in DMSO were diluted to 1.0 ml using ethanol to attain the concentrations 10-50 µg/ml, and were added with DPPH (200 µM, prepared in 95% ethanol). An equal amount of DMSO+ethanol was added to the control. All the tests were performed using triplicate aliquots. The decrease in the absorbance of test compounds was read at 515 nm after 20 min incubation in dark using spectrophotometer (Shimadzu UV-1800) and the percentage inhibition was calculated by using the formula:

\[
\% \text{ scavenging activity} = \frac{A - B}{A} \times 100
\]

Where A is a control absorbance = the measurement of DPPH solution without extract while B is a sample absorbance = the measurement of DPPH solution with the extract. Ascorbic acid and butylated hydroxytoluene (BHT) were used as standard drugs.

Statistical analysis

Antioxidant activity (DPPH assay) of methanol extract of martynia annua seeds were performed in triplicate (n= 3). The presented results were mean±SE (Standard Error). For calculation of EC50, the data were analyzed by Non Linear Regression followed by effector/v/s response analysis on Graphpad Prism 5.0.

RESULTS AND DISCUSSION

GC-MS: The GC-MS chromatogram is shown in fig. 1. As it is difficult to find out or isolate individual component in their purest form, the chromatogram can be used as a fingerprint for the identification of the herbal drug. Different phytochemicals corresponding to different retention time can be predicted and hence these are enlisted in table 1. The higher fatty acids and their esters are dominating. Hence this extract can be a good surfactant which helps to reduce the surface tension of the solvent; GC-MS chromatogram of methanol extract of martynia annua seeds (fig. 1) shows 17 peaks, indicating the presence of 17 phytoconstituents. RT value, peak area, peak area %, molecular formula and compound name of each peak was shown in table 1.

| Compound name | Molecular formula | Peak area % | Peak height | Peak area | RT(min) |
|---------------|------------------|-------------|-------------|----------|---------|
| Palmitic acid | C15H31O2         | 10.83%      | 210369      | 6.67     | 25.47   |
| Hexadecanoic acid, methyl ester | C16H33O2 | 9.17%      | 175744       | 4.08     | 12.36   |
| Octadecadienoic acid, methyl ester | C18H34O2 | 5.87%      | 329243      | 5.12     | 13.17   |
| 2,6,10-trimethyl-12-octadecenoyl[9,10,12,13]tetradec-2-ene | C22H40O | 5.12%      | 912316      | 5.80     | 12.36   |
| Cycloisooctadecene,8,9-dihydro-9-formyl | C20H34O2 | 5.08%      | 329243      | 5.12     | 13.17   |
| 2-Propenoic acid, [3-[4-methoxyphenyl]-ethyl ester | C11H10O2N = 2 | 5.08%      | 100000      | 5.12     | 13.17   |
| 2,6,10-Dodecatrione, 3[E],7[E],11-trimethyl-1-methoxy- | C22H34O2 | 5.08%      | 329243      | 5.12     | 13.17   |
| Heptadecanoic acid, methyl ester- | C17H34O2 | 4.08%      | 210369      | 6.67     | 25.47   |
| Palmitic acid | C15H31O2         | 10.83%      | 210369      | 6.67     | 25.47   |
| 8,11-Octadecadienoic acid, methyl ester | C20H34O2 | 5.87%      | 329243      | 5.12     | 13.17   |
| 12-Methyl-E,E-2,13-octadecadien-1-ol | C22H34O2 | 5.08%      | 329243      | 5.12     | 13.17   |
| 9,12-Octadecadienoic acid[ZZ] | C22H34O2 | 5.08%      | 329243      | 5.12     | 13.17   |
| 9,12-Octadecadienoic acid, [ZZ]-2,3-dihydroxypropyl ester | C22H34O2 | 5.08%      | 329243      | 5.12     | 13.17   |

GC-MS result showed that the first major percentage compound was 12-Methyl-E,E-2,13-octadecadien-1-ol (11.48%) which have been reported to have anticonvulsant, antibacterial, anti-inflammatory, anti-allergic, allergenic, antiseptic, and anti-salmonella activity [19]. The second major percentage compound was Palmitic acid (10.83%) which possesses antimicrobial, anti-inflammatory, anti-nociceptive, and anticancer activity [20]. The third major percentage compound was 8, 11-Octadecadienoic acid, methyl ester (9.77%) which have no biological activity reported. Isopropyl linoleate was the fourth major compound with peak area percentage of 81.18%, it possesses antioxidant, antimicrobial, and anticancer activity [22]. The fifth major percentage compound was 9,12-Octadecadienoic acid[ZZ]-7(9.72%) which have been reported to have anti-inflammatory, anti-adherent vegetable, and nematicide activity [23, 24]. 9,12-Octadecadienoic acid,[ZZ]-2,3-dihydroxypropyl ester possesses antizecmic, hypcholesterolemic,
nematicide, and hepatoprotective [25]. 9,12-Octadecadienoic acid,[Z,Z]-2,3-dihydroxypropyl ester is fatty acid and have been reported to have antioxidant, antimicrobial activity [26]. Some of minor percentage compounds have been reported to have biological activities such as 2-Propenoic acid, 3-[4-methoxyphenyl]-, ethyl ester possesses antimicrobial activity [27]; Flavone possesses antioxidant, hypocholesterolemic, and androgenic activity [28]; Pentadecane has antioxidant and antibacterial activity [29]. GC-MS result showed that the 
*martynia annua* seeds contain bioactive compounds that have medical importance like antioxidant activity.

GC-MS result showed the presence of seven of Fatty acids or their esters: 2-Propenoic acid,3-[4-methoxyphenyl]-ethyl ester(5.80%), Hexadecanoic acid, methyl ester (5.87%), Palmetic acid (10.83%), 8,11-Octadecadienoic acid, methyl ester (9.77%), 9,12-Octadecadienoic acid [Z,Z]-7.92%, Isopropyl linoleate (8.18%), 9,12-Octadecadienoic acid,[Z,Z]-2,3-dihydroxypropyl ester (6.67%) with retention time 15.62, 17.02, 18.05, 18.68, 20.67, 23.17 and 25.47 respectively.

**HPTLC**

The various HPTLC chromatograms at different sample size (2 µl and 5 µl) and at a different wavelength (254 nm and 366 nm) and after derivatization (540 nm) have been shown in fig. 2 to 9. Initially, the HPTLC developed plate was photo-documented at white light, at 254 nm and 366 nm (fig. 2) and scanned at 254 nm and 366 nm. The plates were derivatized using 10% methanolic sulphuric acid and photo-documented at white light and at 366 nm (fig. 3) and scanned at 540 nm.

![Fig. 1: GC MS chromatogram of methanol extract of *martynia annua* seeds](image1)

![Fig. 2: HPTLC chromatogram (a) white light (b) at 254 nm (c) at 366 nm for different size T1: 2 µl, T2: 5 µl](image2)

![Fig. 3: HPTLC chromatogram after derivatization (a) white light (b) at 366 nm for different size T1: 2 µl, T2: 5 µl](image3)
For 2 µl sample size, six peaks were detected and the highest peak area 28.10 % appears at \( R_f = 0.105 \) when the developed plate was scanned at 254 nm, but at the UV wavelength 366 nm, the number of peaks reduced to four and highest area (62.70 %) covered at peak maxima \( R_f = 0.123 \). After derivatization, the number of peaks increases to eight and highest peak get shifted to \( R_f = 0.426 \) with percentage area = 23.21 % (table 2).

When the sample size is increased to 5 µl, the highest peak area (25.01 %) appears at \( R_f = 0.439 \) for 254 nm. When developed plate scanned at 366 nm, the number of peaks observed to be nine and at \( R_f = 0.126 \) highest peak area (48.33 %) observed. After derivatization and derivatized plate scanned at 540 nm, the number of peaks increased to eleven with highest peak area (26.72 %) at \( R_f = 0.473 \).
Table 2: $R_f$ values and area % of HPTLC chromatogram of methanol extract of *martynia annua* seeds, size: 2 µl

| Peak no. | 254 nm ($R_f$) max | Area % | 366 nm ($R_f$) max | Area % | 540 nm (after derivatization) ($R_f$) max | Area % |
|----------|---------------------|--------|---------------------|--------|----------------------------------------|--------|
| 1        | 0.105               | 28.10  | 0.123               | 62.70  | 0.094                                  | 9.01   |
| 2        | 0.252               | 13.99  | 0.406               | 8.34   | 0.303                                  | 62.70  |
| 3        | 0.313               | 9.74   | 0.442               | 22.94  | 0.345                                  | 11.20  |
| 4        | 0.373               | 10.91  | 0.677               | 6.01   | 0.426                                  | 23.21  |
| 5        | 0.440               | 22.29  | 0.474               | 17.68  | 0.511                                  | 12.25  |
| 6        | 0.824               | 14.97  |                     |        | 0.582                                  | 6.64   |
| 7        |                     |        |                     |        | 0.665                                  | 6.33   |

Fig. 7: HPTLC densitometric chromatogram at 254 nm, size: 5 µl

Fig. 8: HPTLC densitometric chromatogram at 366 nm, size: 5 µl

Fig. 9: HPTLC densitometric chromatogram (after derivatization) at 540 nm, size: 5 µl
### Table 3: Rf values and area % of HPTLC chromatogram of methanol extract of *martynia annua* seeds, size: 5 µl

| Peak No. | 254 nm | 366 nm | 540 nm (after derivatization) |
|----------|--------|--------|-------------------------------|
|          | Rf max | Area % | Rf max | Area % | Rf max | Area % |
| 1        | 0.105  | 23.38  | 0.126  | 48.33  | 0.097  | 6.85   |
| 2        | 0.256  | 18.64  | 0.229  | 6.97   | 0.182  | 2.87   |
| 3        | 0.318  | 11.87  | 0.321  | 5.67   | 0.215  | 3.21   |
| 4        | 0.369  | 12.17  | 0.366  | 4.50   | 0.305  | 9.64   |
| 5        | 0.439  | 25.01  | 0.405  | 6.23   | 0.345  | 8.62   |
| 6        | 0.494  | 8.93   | 0.440  | 18.64  | 0.421  | 19.30  |
| 7        | 0.485  | 3.44   | 0.485  | 3.44   | 0.473  | 26.72  |
| 8        | 0.676  | 5.31   | 0.502  | 5.31   | 0.577  | 6.85   |
| 9        | 0.740  | 0.90   | 0.740  | 0.90   | 0.661  | 6.10   |
| 10       |        |        | 0.842  | 1.25   |

### DPPH free radical scavenging assay

Fig. 10 showed the DPPH scavenging activity of various concentrations methanol extract of *martynia annua* seeds. Increase extract concentration leads to increase antioxidant activity (DPPH scavenging activity) and maximum percentage inhibition was found in the extract has 50 µg/ml concentration. EC50 is a concentration of drug or extract required to obtain a 50% antioxidant effect. Table 4 showed EC50 values of methanol extract of *martynia annua* seeds and two standard drugs: ascorbic acid and BHT (butylated hydroxytoluene). Lowest EC50 value means highest antioxidant activity.

Sample which have EC50 lower than 50 µg/ml is a very strong antioxidant, and 50-100 µg/ml is a strong antioxidant, and 101-150 µg/ml is a medium antioxidant while a weak antioxidant with EC50>150 µg/ml [30]. EC50 value of extract was found 44.1±1.1 µg/ml while EC50 of ascorbic acid and BHT were found 11.5±0.6 µg/ml and 12.7±0.4 µg/ml respectively.

### Table 4: EC50 value of DPPH free radical scavenging of extract

| EC50 µg/ml          |          |          |
|---------------------|----------|----------|
| Ascorbic acid (standard) | 11.5±0.6 |          |
| BHT (standard)      | 12.7±0.4 |          |
| *Martynia annua* extract | 44.1±1.1 |          |

Values were obtained as mean±SE (Standard Error), n = 3.

### CONCLUSION

The GC-MS analysis showed the presence of saturated and unsaturated free fatty acids, esters of fatty acids, flavones, alkaloids, aldehydes and ketones whereas in HPTLC different peaks at different UV-lights before and after derivatization were observed. Methanol extract of *martynia annua* seeds exhibited very good inhibition percentage (74%) of DPPH scavenging activity at concentration 50 µg/ml.

### AUTHORS CONTRIBUTIONS

Ali Alrabie, Ola Basa‘ar, and Inas al-qadsy carried out the experiment and wrote the manuscript. Dr. Mazahar Farooqui, the research supervisor conceived the original idea, supervised the project and corrected the scientific content of the manuscript.

### REFERENCES

1. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health perspect 2001;109 Suppl 1:69-75.
2. Revishankar B, Shukla VJ. Indian system of medicine: a brief profile. Afr J Tradit Complement Altern Med 2007;4:319-37.
3. Harish BB, Mohana LS, Saravana KA. Studies on phytochemical and anticonvulsant property of *Martynia annua* linn. Int J Phytopharmacol 2010;1:82-6.
4. Zarina A, Sumayya S, Shaista N. Antioxidant activities and phytochemical screening of *Martynia annua* seeds extract. Biosci Biotech Res Asia 2017;14:1363-9.
5. Prakash P, Prabhakar S, Ramchandra G, Ashish G, Ajag S, Nitin N, et al. Formulation and evaluation of herbal effervescent granules incorporated with martynia annua extract. J Drug Discovery Ther 2013;1:54-7.

6. Eswareiah MC, Eswaruud M, Kumar KP, Kumar PS, Kumar TT. An overview on Martynia Annua L. Int J Pharm Res Dev 2013;5:113-8.

7. Ashwani KD, Bhawna C, Sanjeev KM. Martynia annua L: a review on its ethnombotany, phytochemical and pharmacological profile. J Pharmacogn Phytchem 2013;1:135-40.

8. Renato B, Luciana GW, Gisely CL, Joao CPD. Quality control and TLC profile data on selected plants species commonly found in the Brazilian market. Rev Bras Farmacogn Braz J Pharmaco 2012;22:1111-8.

9. Elangovan NM, Dhanarajan MS, Elangovan I. Preliminary phytochemical screening and HPTLC fingerprinting profile of leaf extracts of Moringa oleifera and Phyllanthus emblica. Int Res J Pharm Biocsi 2015;2:32-40.

10. Shakila R, Ganesan R, Arul AS, Duraipandiyan V. Analytical and HPTLC studies on Innovations Pharm Biol Sci 2017;4:28-35.

11. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Brazilian J Med Biol Res 2000;33:179-89.

12. Gayathri G, Saraswathy A, Vijayalakshmi K. HPTLC fingerprint profile of Bauhinia variegata Linn. leaves. Asian Pacific J Trop Dis 2012;2 Suppl 1:521-5.

13. Murugesan S, Bhuvaneswari S. HPTLC fingerprint profile of methanol extract of the marine red alga portiera hornemannii (Lyngbye) [Silva]. Int J Adv Pharma 2016;5:61-5.

14. Shulamithi R, Sharranya M, Tejawini R, Kiranmai M. Standardization and quality evaluation of herbal drugs. IOSR J Pharm Biol Sci 2016;11:89-100.

15. Anjoo K, Ajay KS. HPTLC fingerprint profile of extracts from dried aerial parts of Bryophyllum pinnatum in different solvents. Pharmacogn J 2010;2:24-31.

16. Pravin HN, Joseph K, Aruna J, Vilasrao K. Future trends in the standardization of herbal drugs. J Appl Pharm Sci 2012;2:38-44.

17. Reich E, Schibli A. High-performance thin layer chromatography for the analysis of medicinal plants. 1st ed. Thieme medical publisher, Inc. The American, Ny; 2007.

18. Sreejayan N, Rao MN. Free radical scavenging activity by curcuminoids. Arzneimittelforschung 1996;46:169-71.

19. Chandana NDML, Jaya PRP, Kondammagari S, Borelli DPR, Nannepaga JS. Phytoconstituents profile of citória ternatae by GC-MS and its age-related anticholinergic activity against aluminum and restraint stress. Int Res J Pharm 2018;9:38-44.

20. Mustapha NA, Runner RTM. GC-MS analysis and preliminary antimicrobial activity of Albizia adiantifolia (Schumach) and Pterocarpus angolensis (DC). Medicines 2016;3:1-9.

21. Brinda C, Mohan KR. Evaluation of the antioxidant, antibacterial activity of ethanolic extract in the leaves of Combretum albidum and gas chromatography-mass spectrometry analysis. Asian J Pharm Clin Res 2016;9:325-9.

22. Rajendran P, Bharathidasan R, Sureshkumar K. GC-MS analysis of phyto-components in raw and treated sugarcane juice. Int J Curr Microbiol Appl Sci 2017;6:5-16.

23. Sheeba GD, Viswanathan P. GC-MC analysis of phyto-components in Spermacoce articularis L. f. leaf. Res Pharm 2014;4:1-7.

24. Nisha, Pasaumarti BR. Gas chromatography-mass spectrometry analysis for identification of bioactive compounds in selected genotypes of Trigonella foenum-graecum L. Pharma Innovation 2018;7:929-39.

25. Padmasheer MS, Roopa B, Ashwathanarayana R, Raja N. Antibacterial properties of Ipomoea staphyllina roem and schult. Plant extracts with comparing its preliminary qualitative phytochemical and quantitative GC-MS analysis. Trop Plant Res 2018;5:349-69.

26. Ali A, Javed A, Shoibah A. GC-MS analysis and antifungal activity of methanolic root extract of Chenopodium album against Scelotium rolfsii. Planta Daninha 2017;35:1-8.

27. Karuppasamy R, Veerabahu RM. GC-MS analysis of bioactive components of Myxopyrum serratum A. W. hill (Oleaceae). Int J Pharm Sci Rev Res 2016;38:30-5.

28. Vardhini SP, Sivaraj C, Himanshu R, Arumugam P. GC-MS, photochemical analysis and antioxidant activities of fruit pulp of Aegle marmelos (L.) correa. Eur J Biomed Pharm Sci 2018;5:569-74.

29. Konovalova O, Gergel E, Herhel V. GC-MS analysis of bioactive components of Shepherdia argentea (Pursh.) nutt. From Ukrainian. Pharma Innovation J 2013;2:7-12.

30. Siti K, Grace N, Irsa F. Antioxidant activities from various extracts of different parts of Kelkai (Stenochlaena palustaris) grown in central Kalimantan-Indonesia. Asian J Pharm Clin Res 2016;9 Suppl 1:215-9.