IJPSR (2014), Volume 5, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 28 February 2014; received in revised form, 10 April 2014; accepted, 24 May 2014; published 01 September 2014

ISOLATION AND CHARACTERIZATION OF THE CHEMICAL CONSTITUENTS OF *STELLARIA MEDIA* LINN.

Disha Arora and Anupam Sharma *

University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh - 160014, Punjab, India.

Keywords:

Stellaria media, Caryophyllaceae, 2, 4, 5, 7-tetramethyloctane, 6-methyl heptyl-3′-hydroxy-2′-methylpropanoate, 2, 2, 4-trimethyloctan-3-one

Correspondence to Author:
Anupam Sharma

University Institute of Pharmaceutical Sciences Panjab University, Chandigarh - 160014, Punjab, India.

E-mail: ans1959@rediffmail.com

ABSTRACT: The studies on the constituents of aerial parts of *Stellaria media* Linn. (Caryophyllaceae) have led to the isolation and characterization of three new compounds ─ (1), (2), and (3). Structure elucidation of the new compounds was carried out using ^{1}H NMR, ^{13}C NMR, Mass spectrometry, together with other complementary techniques (UV and IR). The major findings of the study include: Compound 1 was separated as a white amorphous solid from petroleum ether extract of *S. media*, Methanol extract of *S. media* was subjected to bioactivity-guided fractionation, and Compounds 2 and 3 were isolated, and characterized by different spectral studies. The interpretation of the three compounds include: compound 1 interpreted as 2, 4, 5, 7-tetramethyloctane, compound 2 as - 6-methyl heptyl-3′-hydroxy-2′-methyl propanoate; and compound 3 as - 2, 2, 4-trimethyloctan-3-one.

INTRODUCTION: Plants of the genus *Stellaria* (Caryophyllaceae) are annuals or perennials, distributed in the cold and temperate regions. The genus consists of about 120 species of flowering plants of the family Caryophyllaceae [1]. About 23 species occurring in India [2] are reputed for their medicinal properties. Among these, *S. media* seems to possess wide medicinal potential. The plant has been used traditionally in the treatment of mental tension and inflammations of the digestive, renal, respiratory, and reproductive tracts [3]. The plant is also reported to be anticancer [4], antipyretic, anti-inflammatory [5], antibacterial, antifungal [6, 7], anxiolytic [8].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(9).3669-73

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(9).3669-73

Phenolic acids, flavonoids [9, 10], triterpenoid saponins [11], a pentasaccharide [12], lipids [13] and aqueous constituents [14] have been reported from the genus Stellaria. The study described in this paper was, therefore, undertaken to further examine the phytochemical profile of this plant. Bioactivity-guided fractionation of the anxiolytic methanol extract has led to the isolation of three compounds 1-3. Structures of the compounds were established by spectral studies.

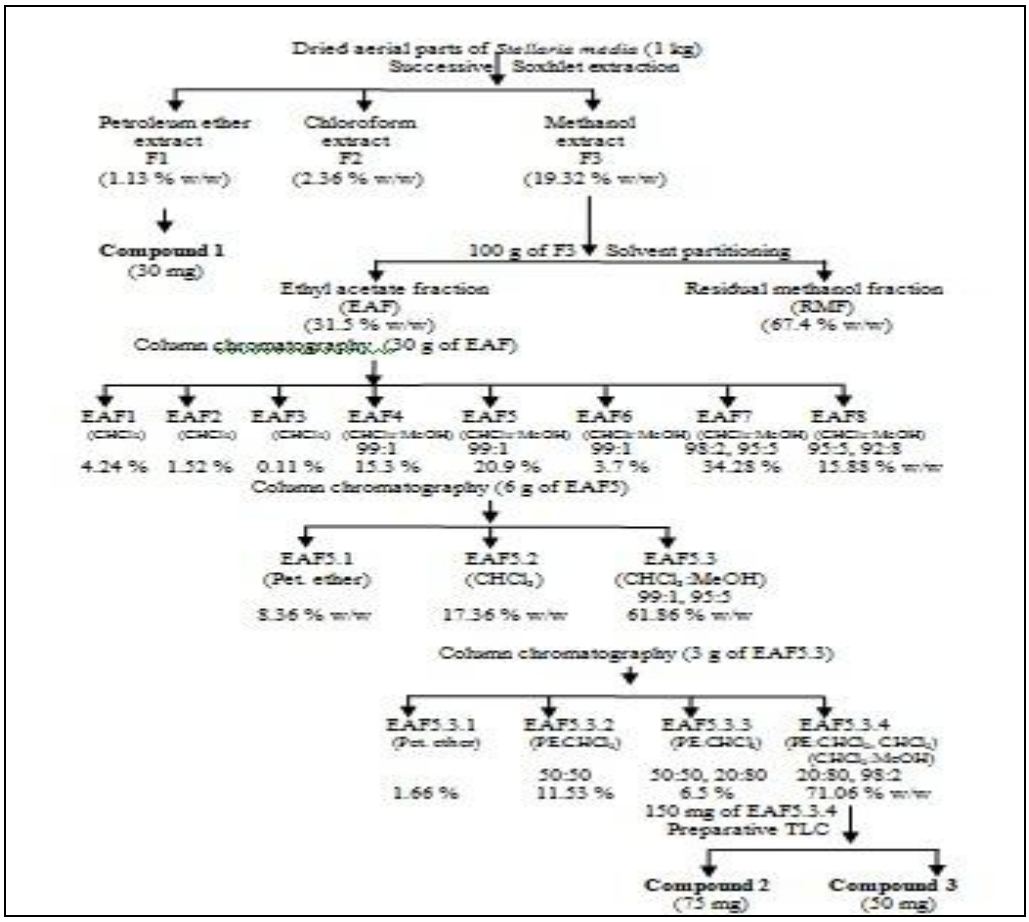
MATERIALS AND METHODS:

General Experimental Procedures: TLC plates (silica gel 60 F_{254}) were purchased from Merck (Darmstadt, Germany). Column chromatography was carried out on silica gel 60, E. Merck, mesh size 60-120, and 230-400. The following instruments at National Institute of Pharmaceutical Education and Research, Mohali were used: IR spectrophotometer (Multi spoke FT-IR synthesis monitoring system, Perkin-Elmer, Germany); mass spectrometer (Finnigan, MAT, LCQ, USA)

equipped with a pneumatically-assisted atmospheric-pressure chemical ionization (APCI). ^{1}H NMR and ^{13}C NMR spectra were obtained on a 400 MHz NMR spectrometer, (Bruker 400, Ultra Shield, ZH079807, Avane, Germany) using $CDCl_3$ as a solvent. Chemical shifts were expressed in parts per million (ppm) relative to tetramethyl silane (TMS) as an internal standard. All solvents used for plant extraction and crude separations were of analytical grade.

Plant Material: Aerial parts of *S. media* were collected from around the University Institute of Pharmaceutical Sciences building, Panjab University, Chandigarh in February 2009. Identity of the plant was confirmed by Dr. H.B. Singh, Head, Raw Materials, Herbarium & Museum at the National Institute of Science Communication and Information Resources, (NISCAIR, CSIR), New Delhi 110 067. A voucher specimen no: NISCAIR/RHMD/Consult/2008-09/1170/202 is deposited in the same herbarium.

Extraction and Isolation: Scheme 1 shows the extraction of *S. media* aerial parts, and isolation of three compounds (1-3).



SCHEME 1: ISOLATION OF COMPOUNDS 1-3

Powdered aerial parts of the plant (1 kg) were subjected to successive Soxhlet extraction by solvents in increasing order of polarity *viz*. petroleum ether (60-80 °C), chloroform and methanol. From the concentrated petroleum ether extract (2 g) of *S. media*, an amorphous material separated after about 48 h, and this was collected by filtration. The residue on the filter paper was washed (3 × 2 ml) with petroleum ether, and finally, air dried to get 30 mg of an amorphous solid.

TLC of the separated material using cyclohexane: toluene (9.5:0.5) showed a single spot (R_f 0.95) when visualized using 0.5% anisaldehyde sulphuric acid spraying reagent. This compound was labeled as 1. Earlier studies carried out by the authors revealed that of the four extracts, only the methanol extract exhibited anxiolytic activity [8]. Repeated bioactivity guided fractionation of methanol extract using solvent partitioning and column chromatography led to the separation of a subfraction EAF5 (Ethyl acetate fraction 5) which

was found to be responsible for the antianxiety activity of the methanol extract [15]. The present study was carried out to separate the components in EAF5. The TLC profile of EAF5 showed the presence of two components (R_f 0.73 and 0.78, respectively). These two compounds were separated using preparative TLC. From 150 mg of EAF5, two compounds were obtained, namely 2 (75 mg) and 3 (50 mg).

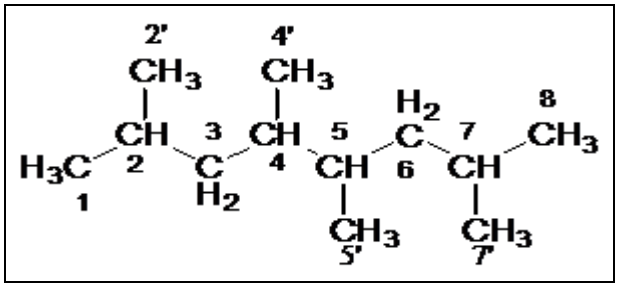
Characterization of 2, 4, 5, 7-tetramethyloctane (1): White amorphous solid; m. p. 48-50 °C; UV_{max} (MeOH) 180 nm; IR (KBr) 722.9, 1376.2, 1461.9, 2852.5, 2920.8 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.84 (br, 4H, 2 x -C*H*$_2$), 0.86 (br, 2H, 2 x -C*H*), 1.25 (br, 18H, 6 x -C*H*$_3$), 1.55 (m, 2H, 2 x -C*H*), ppm; ^{13}C-NMR ($CDCl_3$) δ 14.09 (2 × -CH-*C*H$_3$), 22.67 [2 × -CH (*C*H$_3$)$_2$], 29.68 [2 × -*C*H (CH$_3$)$_2$], 31.92 (2 ×-*C*H-CH$_3$), 37.10 (-*C*H$_2$) ppm; MS (APCI) *m/z* 170 $[M]^+$ ($C_{12}H_{26}$), 172 $[M+2]^+$, 127 [M - CH (CH$_3$)$_2$]$^+$, 85 [M – CH (CH$_3$) (CH$_2$)-CH (CH$_3$)$_2$]$^+$, 57 [M – CH (CH$_3$)-CH (CH$_3$)-CH$_2$-CH (CH$_3$)$_2$]$^+$.

Characterization of 6-methylheptyl-3′-hydroxy-2′-methylpropanoate (2): Yellow semi-solid; m. p. 70-72 °C; UV_{max} (MeOH) 246 nm; IR (KBr) 1090.7, 1157.4, 1262.1, 1378.1, 1458.4, 1727.6, 2857.0, 2924.2, 3426.0 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.80 (m, 2H, -C*H*$_2$), 1.08 [br, 1H, -C*H* (CH$_3$)$_2$], 1.35 (br, 6H, 3 × -C*H*$_3$), 1.52 (m, 2H, -C*H*$_2$), 1.72 [br, IH, -C*H* (CH$_2$OH) (CH$_3$], 1.83 (m, 2H, -C*H*$_2$), 2.07 (m, 2H, -C*H*$_2$), 2.35 (d, 3H, -C*H*$_3$), 2.70 (br, 1H, O*H*), 4.32 (t, 2H, -C*H*$_2$) ppm; ^{13}C-NMR ($CDCl_3$) δ 14.13 [-CH (*C*H$_3$) (CH$_2$OH)], 22.70 [-CH (*C*H$_3$)$_2$], 25.52 [-*C*H$_2$-(CH$_2$)$_2$-CH (CH$_3$)$_2$], 27.09 [-*C*H$_2$-CH$_2$-CH (CH$_3$)$_2$], 29.36 (-*C*H (CH$_3$)$_2$), 29.66 (-*C*H$_2$-CH$_2$-O-C=O), 37.09 (-*C*H$_2$-CH (CH$_3$)$_2$), 50.04 (-*C*H (CH$_3$) (CH$_2$OH), 57.36 (-*C*H$_2$OH), 61.70 (-*C*H$_2$-O-), 185.56 (-*C*=O) ppm; MS (APCI) *m/z* 216 $[M]^+$ ($C_{12}H_{24}O_3$), 217 $[M+1]^+$, 173 [M – CH (CH$_3$)$_2$]$^+$, 145 [M – CH (CH$_3$)$_2$-(CH$_2$)$_2$]$^+$, 131 [M – CH (CH$_3$)$_2$-(CH$_2$)$_3$]$^+$, 103 [M – O-C=O-CH (CH$_3$)-CH$_2$OH]$^+$.

Characterization of 2, 2, 4-trimethyloctan-3-one (3): White crystals; m. p. 76-78 °C; UV_{max} (MeOH) 243 nm; IR (KBr) 1295.2, 1465.3, 1705.4, 2850.9, 2919.4 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.9 (pent, 2H, -C*H*$_2$), 1.20 (br, 15H, 5 × -C*H*$_3$), 1.25 (br, 2H, -C*H*$_2$), 1.63 (m, 2H, -C*H*$_2$), 2.35 (m, 1H, -C*H*) ppm; ^{13}C-NMR ($CDCl_3$) δ 14.12 (-*C*H$_3$), 22.69 (-CH-*C*H$_3$), 24.70 (-*C*H$_2$-CH$_3$), 29.06 [-C (*C*H$_3$)$_3$], 29.24(-*C*H$_2$-CH$_2$-CH$_3$), 29.70 (-*C*H$_2$-(CH$_2$)$_2$-CH$_3$), 31.93 [-*C*H (CH$_3$) (CH$_2$)], 195.56 (-*C*=O) ppm; MS (APCI) *m/z* 170 $[M]^+$ ($C_{11}H_{22}O$), 172 $[M+2]^+$, 141[M – CH$_2$-CH$_3$]$^+$, 113 [M – (CH$_2$)$_3$-CH$_3$]$^+$, 57 [M – C=O-CH (CH$_3$)-(CH$_2$)$_3$-CH$_3$]$^+$.

RESULTS AND DISCUSSION: Compounds 1-3 are being reported for the first time from *S. media*.

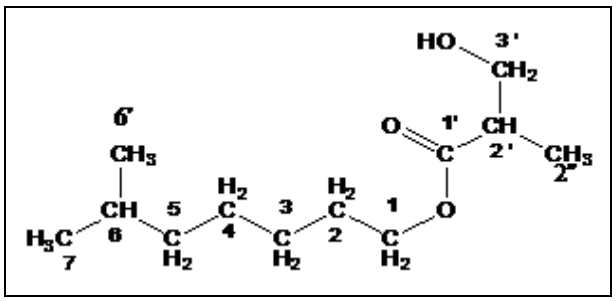
Compound 1 was isolated as white amorphous solid. The mass spectra of compound 1 showed $[M]^+$ at *m/z* 170 suggesting the molecular formula $C_{12}H_{26}$ and $[M+2]^+$ ion peak at *m/z* 172. The loss of 43 molecular units generated an ion at *m/z* 127 indicated the presence of [M - CH (CH$_3$)$_2$] $^+$. Significant ions at *m/z* 85 and 57 correspond to [M – CH (CH$_3$) (CH$_2$)-CH (CH$_3$)$_2$] $^+$ and [M – CH (CH$_3$)-CH (CH$_3$)-CH$_2$-CH (CH$_3$)$_2$] $^+$. The IR spectrum featured bands at 722.9 cm^{-1} (long chain band), 1376.2 cm^{-1} (CH_3 bending), and 1461.9 cm^{-1} (CH_2 bending). Aliphatic C-H stretch featured at 2852.5 and 2920.8 cm^{-1}. In the ^{1}H NMR spectrum, signals appeared at δ 0.84 (br, 4H, 3-CH_2 & 6-CH_2), 0.86 (br, 2H, 2-CH & 7-CH), 1.25 (br, 18H, 1-CH_3, 2′-CH_3, 4′-CH_3, 5′-CH_3, 7′-CH_3 & 8-CH_3), 1.55 (m, 2H, 4-CH & 5-CH), ppm. In the ^{13}C NMR spectrum, the signals showed at δ 14.09 (2C, 4′-CH_3 & 5′-CH_3), 22.67 (2C, 2′-CH_3 & 7′-CH_3), 29.68 (2C, 2-CH & 7-CH), 31.92 (2C, 4-CH & 5-CH), 37.10 (2C, 3-CH_2 & 6-CH_2) ppm. These data suggested the structure of this compound to be 2, 4, 5, 7-tetramethyloctane.



COMPOUND 1

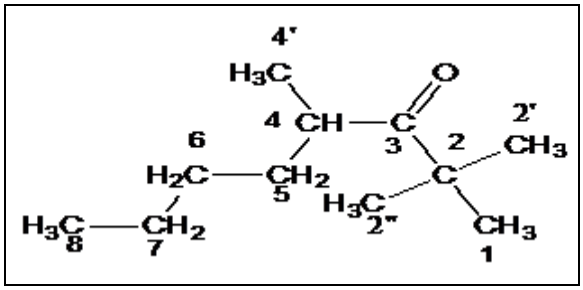
Compound 2 was isolated as yellow gummy solid. The mass spectrum of compound 2 indicated an $[M]^+$ at *m/z* 216 suggesting the molecular formula $C_{12}H_{24}O_3$ and $[M+1]^+$ at *m/z* 217. The loss of 43 molecular units generated an ion at *m/z* 173 indicated the presence of [M – CH (CH$_3$)$_2$] $^+$. Significant ions at m/z 145, 131 and 103 correspond to [M – CH (CH$_3$)$_2$-(CH$_2$)$_2$] $^+$, [M -CH

$(CH_3)_2$-$(CH_2)_3$] $^+$ and [M – O-C=O-CH (CH_3)-$CH_2OH]^+$ respectively. The IR spectrum showed bands at 1090.7 and 1157.4 cm^{-1} for C-O stretching of alcohol and ester respectively. OH stretch featured at 3426.0 cm^{-1} and C=O stretch featured at 1727.6 cm^{-1}. In the ^1H NMR spectrum, signals appeared at δ 0.80 (m, 2H, 5-CH_2), 1.08 (br, 1H, 6-CH), 1.35 (br, 6H, 7-CH_3 & 6′-CH_3), 1.52 (m, 2H, 4-CH_2), 1.72 (br, IH, 2′-CH), 1.83 (m, 2H, 3-CH_2), 2.07 (m, 2H, 2-CH_2), 2.35 (d, 3H, 2″-CH_3), 2.70 (br, 1H, 3′-OH), 4.32 (t, 2H, 3′-CH_2) ppm. Its ^{13}C NMR signals appeared at δ 14.13 (1C, 2″-CH_3), 22.70 (2C, 7-CH_3 & 6′-CH_3), 25.52 (1C, 3-CH_2), 27.09 (1C, 4-CH_2), 29.36 (1C, 6-CH), 29.66 (1C, 2-CH_2), 37.09 (1C, 5-CH_2), 50.04 (1C, 2′-CH), 57.36 (1C, 3′-CH_2), 61.70 (1C, 1-CH_2), 185.56 (1C, 1′>C=O) ppm. In light of these observations, the structure of 2 was elucidated as 6-methyl heptyl-3′-hydroxy-2′-methyl propanoate.



COMPOUND 2

Compound 3 was isolated as white crystals. The mass spectrum of compound 3 showed a molecular ion peak at *m/z* 170 calculated for $C_{11}H_{22}O$ and $[M+2]^+$ at *m/z* 172. The loss of 113 molecular units from $[M]^+$ generated an ion at *m/z* 57 indicated the presence of tertiary carbon, correspond to [M – C=O-CH (CH_3)-$(CH_2)_3$-$CH_3]^+$. Significant ions at *m/z* 141 and 113 indicated the presence of [M – CH_2-$CH_3]^+$ and [M – $(CH_2)_3$-$CH_3]^+$. The IR spectrum showed bands at 1295.2 and 1705.4 cm^{-1} for C-C=O-C bending and C=O stretching. In the ^1H NMR spectrum, signals appeared at δ 0.9 (pent, 2H, 6-CH_2), 1.20 (br, 15H, 1-CH_3, 2′-CH_3, 2″-CH_3, 4′-CH_3 & 8-CH_3), 1.25 (br, 2H, 7-CH_2), 1.63 (m, 2H, 5-CH_2), 2.35 (m, 1H, 4-CH) ppm. In the ^{13}C NMR spectrum, signals appeared at δ 14.12 (1C, 8-CH_3), 22.69 (1C, 4′-CH_3), 24.70 (1C, 7-CH_2), 29.06 (3C, 2′-CH_3, 2″-CH_3 & 1-CH_3), 29.24 (1C, 6-CH_2), 29.70 (1C, 5-CH_2), 31.93 (1C, 4-CH), 195.56 (1C, 3 >C=O) ppm. These data led to characterize compound 3 as 2, 2, 4-trimethyloctan-3-one.



COMPOUND 3

Studies on the constituents of aerial parts of *Stellaria media* Linn. have led to the isolation and characterization of three compounds ─ 2, 4, 5, 7-tetramethyloctane, 6-methylheptyl-3′-hydroxy-2′-methylpropanoate, 2,2,4-trimethyloctan-3-one.

CONCLUSION: The present study involved the isolation and characterization of three new compounds - Alkane (1) was separated as a white amorphous solid from petroleum ether extract of *S. media.* Bioactivity-guided fractionation of methanol extract of *S. media* ultimately led to the isolation of two compounds ― an ester (2) and a ketone (3). All the three compounds were reported for the first time in *S. media* Linn.

ACKNOWLEDGEMENT: This work was funded by the University Grants Commission (UGC).

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Ankei T: Habitat gradient and reproductive habits of the seven Stellaria species in Japan. Bot Mag Tokyo 1982, 94: 35-48.
2. Anonymous. The Wealth of India, Raw materials, Publications and Information Directorate, Vol X, CSIR, New Delhi 1976: 39-40.
3. Shan Y, Zhou J, Zhao HG, Feng X, Dong Y and Xia B: Amino acid and mineral composition of *Stellaria media*. Chem Nat Compd 2010; 46: 667-68.
4. Chon SU, Heo BG, Park YS, Kim DK and Gorinstein S: Total phenolics level, antioxidant activities and cytotoxicity of young sprouts of some traditional Korean salad plants. Plant Foods Hum Nutr 2009; 64: 25-31.
5. Morita H, Kayashita T, Shishido A, Takeya K, Itokawa H and Shiro M: Dichotomins A – E, New Cyclic Peptides from *Stellaria dichotoma* L. var. lanceolata Bge. Tetrahedron 1996; 52: 1165-76.
6. Zhou CC, Sun XB, Liu JY, Luo SQ and Lu CY: Anti-inflammatory effect of α-spinasterol. Acta Pharm Sinica 1985, 20: 257-61.
7. Shinde NV, Himaja M, Bhosale SK, Ramana MV and Sakarkar DM: Synthesis and biological evaluation of Delavayin-C. Ind J Pharm Sci 2008; 70: 827-31.
8. Arora D and Sharma A: Evaluation of anxiolytic activity of *Stellaria media* Linn. extracts in mice. Phcog Comm 2012; 2: 58-61.

9. Kitanov G: Phenolic acids and flavonoids from Stellaria media (L.) Vill. (Caryophyllaceae). Pharmazie 1992, 47: 470-71.
10. Yong-Mei H, Wen-Cai Y, Qian L, Hai-Yan T, Hao W and Hong-Yu D: C-Glycosylflavones from *Stellaria media*. Zhongguo Tianran Yaowu 2006, 4: 420-24.
11. Hodisan V and Sancraian A: Triterpenoid saponins from *Stellaria media* (L.) Cyr. Farmacia 1989; 37: 105-09.
12. Vanhaecke M, Van den Ende W, Lescrinier E and Dyubankova N: Isolation and characterization of a pentasaccharide from *Stellaria media*. J Nat Prod 2008; 71: 1833-36.
13. Pande A, Shukla YN and Tripathi AK: Lipid constituents from *Stellaria media*. Phytochemistry 1995; 39: 709-11.
14. Hu Y, Wang H, Ye W and Liu G: Aqueous constituents from *Stellaria media* (L.) Cyr. Zhongguo Yaoke Daxue Xuebao 2005; 36: 523-25.
15. Sharma A and Arora D: Bioactivity directed separation of an anxiolytic fraction from *Stellaria media* Linn. J Pharm Res 2012; 5: 4629-32.

How to cite this article:
Arora D and Sharma A: Isolation and characterization of the chemical constituents of *Stellaria media* Linn. Int J Pharm Sci & Res 2014; 5(9): 3669-73. doi: 10.13040/IJPSR.0975-8232.5(9).3669-73.