Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Isolation and Characterization of Beta-Sitosterol from Rice Bran

S. K. Raziya[1], Poiba Venkata Rao[2], Kanamala Arun Chand Roby[3], Sreenu Thalla[3], Jada Naga Lakshmi[4], Mohammed Rageeb Mohammed Usman[5], Prabhakar Vishvakarma[6], Bonige Kishore Babu[7*]

[1]Assistant Professor, Department of Chemistry (BS&H) Viskha Institute of Engineering& Technology(A), Narva, Viskhaptnam
[2]Assistant professor, Department of Chemical engineering, Au College of engineering (A), Andhra University vishakapatnam
[3]Assistant Professor, Vignan Pharmacy College (Autonomous), Vadlamudi, Guntur, Andhra Pradesh
[4]Associate Professor, Vignan Pharmacy College (Autonomous), Vadlamudi, Guntur, Andhra Pradesh
[5]Professor and HOD, Department of Pharmaceutics, Krishna Institute, Bijnor, Uttar Pradesh
[6]Associate Professor, Department of Pharmaceutics, Krishna Institute, Bijnor, Uttar Pradesh
[7]Associate Professor, Department of Engineering Chemistry, AUCE(A) Andhra University Visakhapatnam
Email: vjacobkishore@gmail.com

A "treasure house of natural nutrition" is rice bran. Nevertheless, the use of rice bran is frequently disregarded, which has led to nutrient waste. One of the active ingredients in rice bran, sterol, has drawn a lot of interest due to its antibacterial, immunostimulating, antioxidant, anticancer, and hypoglycemic qualities. Finding and describing the bioactive principle in rice bran is the aim of this investigation. Hexane extract of rice bran was subjected to phytochemical screening, which revealed the presence of phytosterol. By combining 10 g of the extract with 200 mL of acetonitrile, phytosterols were separated from the extract. Thin layer chromatography and High Performance Liquid Chromatography (HPLC) were used to analyse and purify the resultant white sterol crystals. The sterol was further investigated using more advanced analytical methods, such as FTIR, 1H-NMR, 13C-NMR, and LC-MS, in order to clarify its structure. The isolated substance was determined to be beta sitosterol via a variety of spectral analyses

and interpretations.

Keywords: Rice bran, Extraction, Isolation, Phytosterols, Beta sitosterol.

1. Introduction

Cereals are an important part of the human diet because of their substantial calorie contribution as well as the vast range of nutrients they offer. Since rice represents a staple food for over 60% of the world's population, it is currently one of the most significant grains from a nutritional and economic standpoint. More precisely, rice is the most widely grown crop in the Asia-Pacific area and is a staple in many developing nations, including Bangladesh, Vietnam, India, and others. [1, 2]

For most people around the world, particularly in Asia, South America, and Africa, rice is a staple diet. The main by-product of rice is rice bran, which is found on the surface of the grain and is separated from it when brown rice is milled. It makes about 5–8% of the total weight of rice and is made up of exocarp, mesocarp, a cross-linked layer, an aleurone layer, and other structural layers [3, 4]. Around 500 million tonnes of rice are produced annually worldwide, producing more than 25 million tonnes of rice bran [5]. In addition to being a plentiful resource, rice bran has a very high nutritious content. 12–16% protein, 12–23% fat, and 23–30% dietary fibre are all present in rice bran. It is also rich in vitamins, minerals, and other nutrients like glutamate, phytic acid, inositol, and phenanthrene [6, 7]. According to ancient Chinese medical books, rice bran also tones blood and qi, boosts appetite, nourishes the bowels, and may be used to cure foot ailments [8]. It is considered a "treasure house of natural nutrition" as a result. However, at the moment, there is a substantial loss of resources due to the extremely low comprehensive utilisation rate of rice bran, with the majority of it being either utilised as chicken feed or thrown away directly [9]. Rice bran has been used to make a wide variety of phytochemical components, including flavonoids, alkaloids, saponins, terpenes, and complex sterols [10].

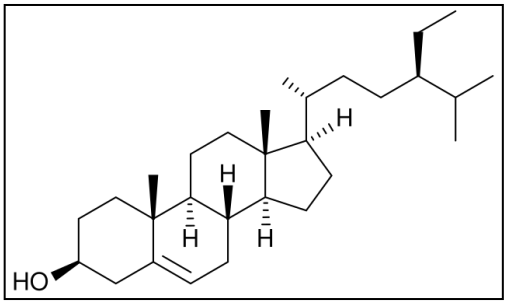


Fig. 1: Structure of (β-sitosterol)

The category of phytosterols, which contains stigmasterol and campesterol in particular, includes β-sitosterol. Similar in structure and function to cholesterol, phytosterols are essential steroid molecules that stabilise the phospholipid bilayers of plant cellular membranes. [11] They constitute a significant class of bioactive components with established bioactivity. In vivo, phytosterols have a number of health advantages, most notably defence against chronic conditions like diabetes, cancer, heart disease, and liver damage. It is important to note that

phytosterols have recently garnered a lot of attention due to their well-known ability to decrease cholesterol [12, 13]. This study's primary goal is to identify and separate this component rice bran because it is a crucial therapeutic compound.

2. MATERIALS AND METHODS

Procurement of and Extraction of Plant Material: We purchased rice bran from the local markets in Meerut, Uttar Pradesh, India. 50 grammes of shade-dried plant material were extracted from rice bran using a Soxhlet apparatus and 350 millilitres of hexane. The extract was then filtered, and a rotary evaporator was used to evaporate the solvent at lower pressure. Using thin-layer chromatography (TLC) with spray reagent, hexane extract was examined for the presence of terpene, and the results were verified by liquid chromatography (LC)-MS analysis. [14, 15]

Preliminary Phytochemical screening:

Liebermann-Burchard test: Extract 1mg was dissolved in chloroform and few drops of acetic anhydride were added to it, followed by concentrated sulphuric acid from the side of the tube. A transient color development from red to blue and finally green indicated the presence of sterol. [16]

Salkowaski Reaction: Extract 1mg was dissolved in 2 ml of chloroform and 2ml of concentrated sulphuric acid was added from the side of the test tube. The test tube was shaken for few minutes. The development of red color in the chloroform layer indicated the presence of sterol. [16]

Isolation of Phytosterols: The hexane extract was mixed with 200 millilitres of acetonitrile to separate the phytosterols. The temperature of the mixture was raised to about acetonitrile's boiling point. Ten to fifteen minutes were spent maintaining this temperature. At this point, a layer of the unwanted gummy substance, which is an insoluble unsaponifiable component, formed at the bottom of the jar; the acetonitrile and sterols dissolved in a clear solution. After this sterol-acetonitrile solution was decanted, white sterol crystals began to form. [17, 18]

The resulting crystals were analysed by Thin layer Chromatography and High Performance Liquid Chromatography (HPLC) for the purification.

TLC: The separated crystals underwent thin-layer chromatography in the solvent system of chloroform: acetone (7:3), and the reagent vanillin-sulfuric acid was sprayed on them to see them. After separating the two spots using preparative CO-TLC and several thin-layer chromatographic runs, the spot with Rf value 0.5 was pooled, the solvent was evaporated, and it was dried. The separation was done using the preparatory glass plates. [19, 20]

High Performance Liquid Chromatography (HPLC): The HPLC system included a Water's 600 controller pump, a Phenomex C18 (250 X 4.60 mm) column, a UV visible detector (wavelength adjustable), a digital analytical weighing balance (Wesnar), a 5µm column, and a Data Ace software (Chromatography workstation).

HPLC Conditions: Prior to use, the mobile phase, which was composed of acetonitrile:methanol (9:1), was filtered through a 0.45µ membrane filter, degassed, and

injected into the column at a flow rate of 0.6 ml/min from the solvent reservoir at a 9:1 v/v ratio. The temperature of the column was 30°C. The run time was 12–15 minutes, and the detection was observed at 210 nm. The injection loop's volume was 20 µl, and the column was equilibrated with the mobile phase flowing through the system for at least 15 minutes before the drug solution was injected. [21-25]

Spectroscopic Studies: More advanced analytical methods, such as FTIR, ^{1}H-NMR, ^{13}C-NMR, and LC-MS, were used to further study the sterol for structure elucidation (Bruker FT-IR Spectrometer, USA). Using $CDCl_3$ as a solvent, 1H and 13C-NMR spectra were recorded at Biospience Laboratory in Bhopal, USA, using a Bruker BioSpin Advance III FT-NMR spectrometer, while LC-MS spectra were recorded at high resolution on a Bruker Aurora M90 (USA) device. [26-30]

3. RESULTS AND DISCUSSION

Tests on rice bran extract revealed the presence of phytosterol. It is believed to be a substance with a steroidal nucleus based on the positive results for steroids. The melting point of the isolated white, crystalline substance that resembled needles was found to be between 145 and 148 °C. Chloroform: acetone (7:3) was chosen for TLC fingerprinting based on these findings. In standard settings, the Rf value of β-sitosterol was between 0.50 and 0.51 under the chromatographic conditions mentioned above. Both isolated beta-sterol and beta-sterol.

HPLC Analysis: One of the most often used methods for assessing the phenolic chemicals found in plants is the HPLC analysis. Because plants are so complex and diverse, it is impossible to characterise every phenolic molecule. For identifying βsitosterol in rice bran, high performance liquid chromatography (HPLC) is an appropriate analytical technique.

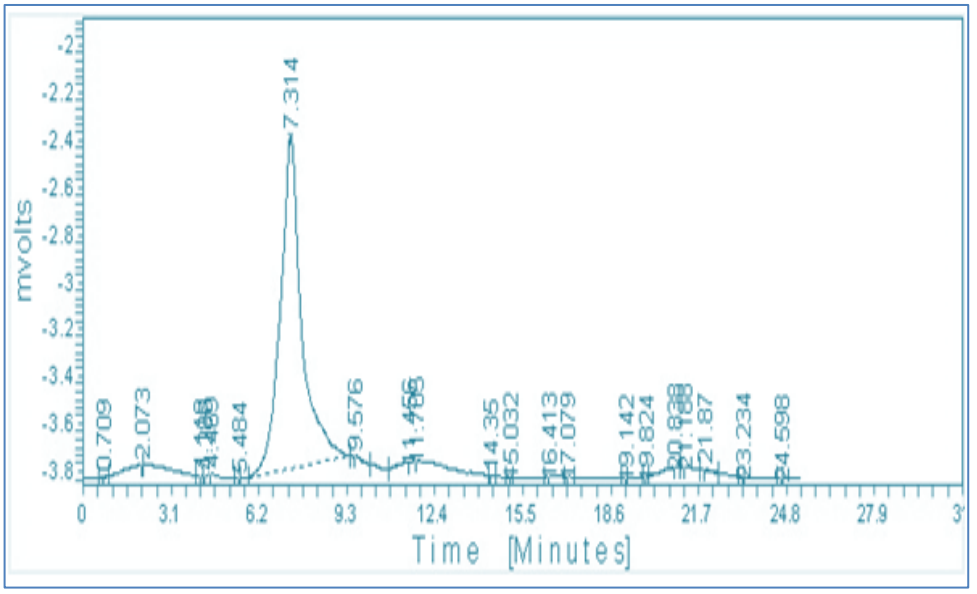


Fig 1: Chromatogram of the Isolated Compound (β-sitosterol)

FTIR Analysis: One method for determining the chemical bonds and functional groups found in plant extracts is Fourier transform infrared (FTIR) spectroscopy. Absorption peaks were observed at 2953.02 cm^{-1} (O-H stretching), 2916.37 cm^{-1} and 2848.86 cm^{-1} (aliphatic C-H stretching), and 1691.57 cm^{-1} (C=C absorption peak) in the IR absorption spectrum (Fig. 3). Other absorption peaks included 1462.04 cm^{-1} (CH_2), 1365.6 cm^{-1} (OH def), and 1031.92 cm^{-1} (cycloalkane).

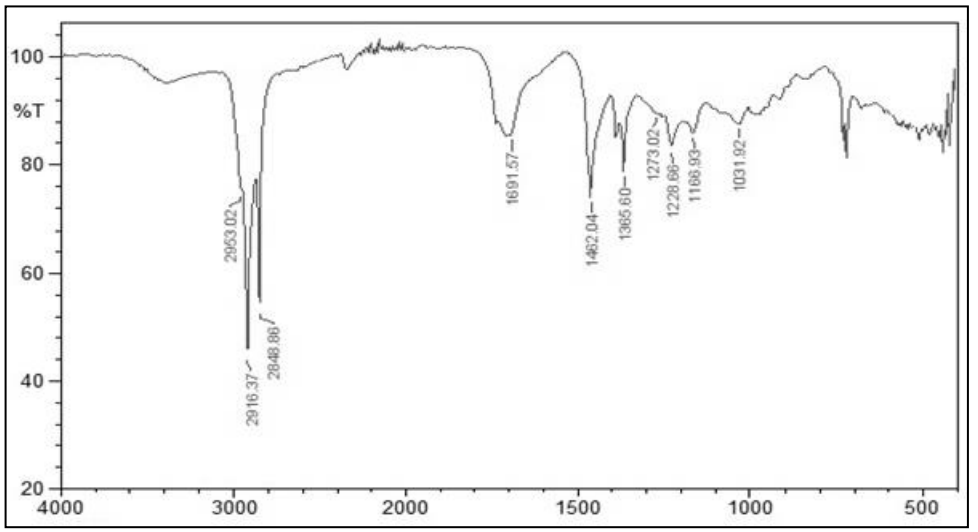
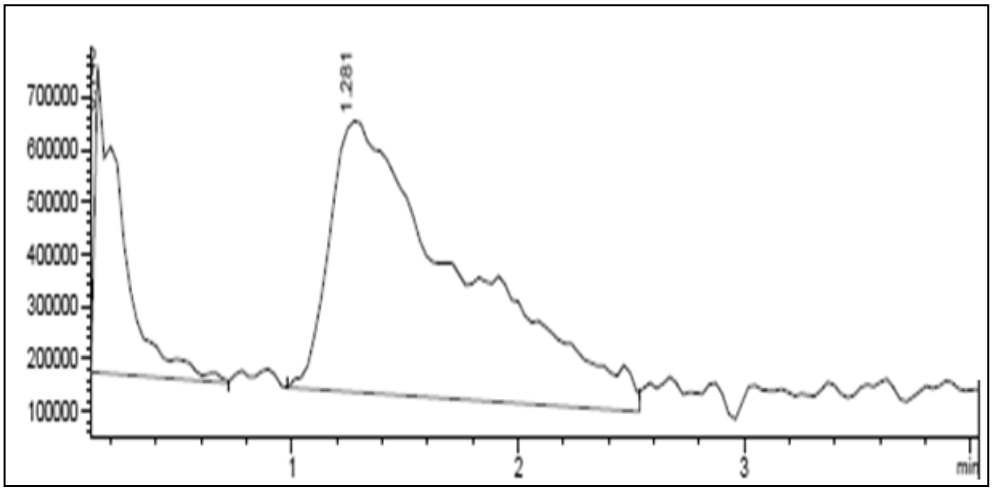


Fig. 2: FTIR Spectra of isolated compound (β-sitosterol)

LC-MS Spectral Analysis: The molecular ion peaks at 413.5 that match the molecular formula $C_{29}H_{50}O$ were visible in LC-MS spectroscopy. Additionally, ion peaks were detected at m/z 397.5, 383.5, 371.1, and 282.3. The molecules exhibited are β-sitosterol, as indicated by the molecular weight and fragmentation pattern.



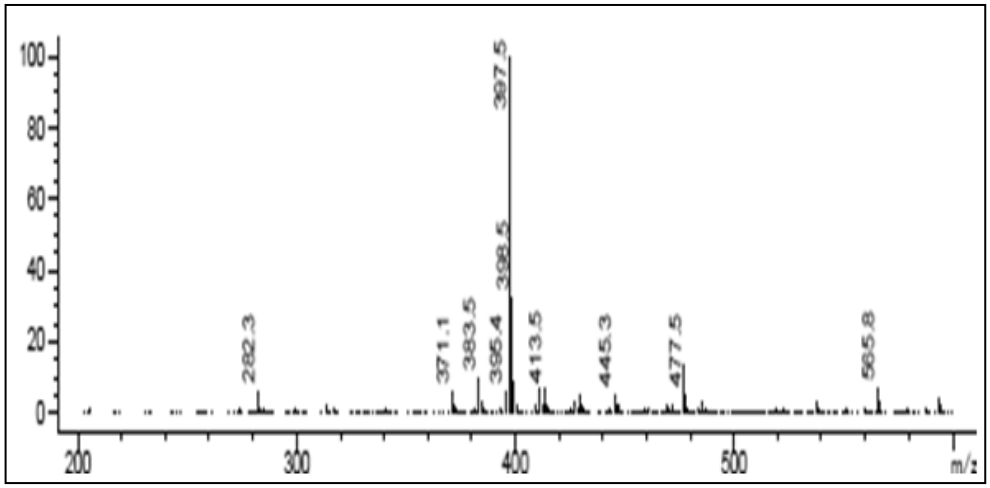


Fig.-3 LCMS Spectra of Isolated Compound (β-sitosterol)

NMR Spectral Analysis:

1HNMR ($CDCl_3$, 400MHz): ^{1}H-NMR has given signals at δ 3.2(1H, m, H-3), 5.26 (1H, m, H-6), 5.19(1H, m, H-23), 4.68(1H,m,H- 22), 3.638(1H, m, H-3), 2.38(1H, m, H-20), 1.8-2.0 (5H, m) ppm Other peaks are observed at δ 0.76-0.89 (m, 9H), 0.91-1.05 (m, 5H), 1.35-1.42 (m, 4H), 0.69-0.73 (m, 3H), 1.8-2.00 (m, 5H), 1.07-1.13 (m, 3H), 1.35-1.6 (m, 9H) ppm.

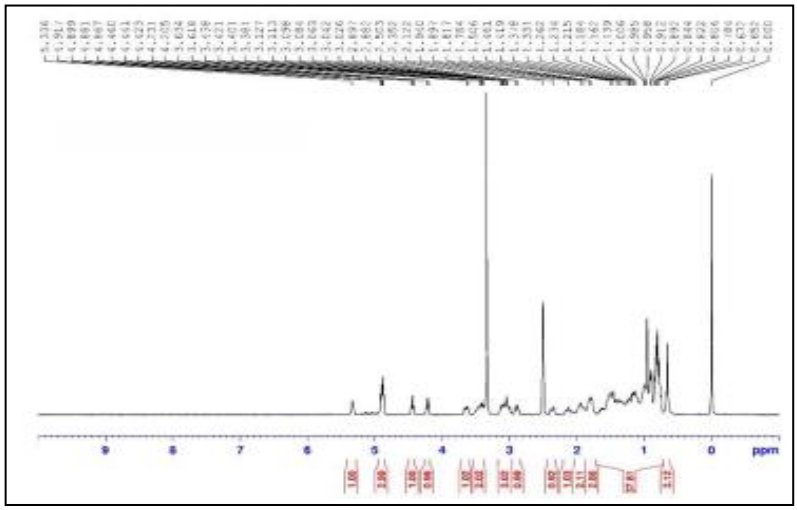


Figure 4: ^{1}H-NMR Spectra of isolated compound ((β-sitosterol)

The above I.R., FTIR, 1HNMR, and LC-MS spectral data and their comparison with those described in the literatures showed the structure of β-sitosterol.

Identification of Compound: Following a variety of spectral analyses, the isolated molecule was determined to be $C_{29}H_{50}O$ beta sitosterol during interpretation by comparing the structure with spectral libraries and chemical testing.

4. CONCLUSION

Although no one chemical has been identified as responsible for Rice bran medicinal benefits, biological studies have demonstrated that beta-sitosterol, a phytosterol, attenuates high cholesterol and heart disease. In addition to treating gallstones, hair loss, bronchitis, migraines, and headaches, it also strengthens the immune system and prevents colon cancer. Beta-sitosterol is used by some men to treat benign prostatic hyperplasia, or BPH, which is an enlarged prostate. Some women use it to treat menopausal symptoms. Rice bran contains significant amount of sitosterol, and further studies needed to investigate the presence of other sterol like beta-sterol which is reported to be found in the plant.

References

1. Prabhakar V, Agarwal S, Chauhan R, Sharma S. Fast dissolving tablets: an overview. International Journal of Pharmaceutical Sciences: Review and Research. 2012;16(1):17.
2. Mandal S, Vishvakarma P, Verma M, Alam MS, Agrawal A, Mishra A. Solanum Nigrum Linn: an analysis of the Medicinal properties of the plant. Journal of Pharmaceutical Negative Results. 2023 Jan 1:1595-600.
3. Vishvakarma P, Mandal S, Pandey J, Bhatt AK, Banerjee VB, Gupta JK. An Analysis of The Most Recent Trends in Flavoring Herbal Medicines in Today's Market. Journal of Pharmaceutical Negative Results. 2022 Dec 31:9189-98.
4. Mandal S, Vishvakarma P, Mandal S. Future Aspects and Applications of Nanoemulgel Formulation For Topical Lipophilic Drug Delivery. European Journal of Molecular & Clinical Medicine. 2023;10(01):2023.
5. Mandal S, Vishvakarma P. Nanoemulgel: A Smarter Topical Lipidic Emulsion-based Nanocarrier. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s481-98.
6. Prabhakar V, Agarwal S, Chauhan R, Sharma S. Fast dissolving tablets: an overview. International Journal of Pharmaceutical Sciences: Review and Research. 2012;16(1):17.
7. Seetharamaiah GS, Chandrasekhara N. Studies on hypocholesterolemic activity of rice bran oil. Atherosclerosis. 1989;78(2-3):219-223.
8. Sukanya SL, Sudisha J, Hariprasad P, Niranjana SR, Prakash HS, Fathima SK. Anti-microbial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. African Journal of Biotechnology, 2009, 8(23).
9. Tanner N, Lichtenberg-Kraag B. Identification and Quantification of Single and Multi-Adulteration of Beeswax by FTIR-ATR Spectroscopy. European Journal of Lipid Science and Technology. 2019;121(12):1-10. https://doi.org/10.1002/ejlt.201900245
10. Thangaraju S, Pulivarthi MK, Natarajan V. Waste from Oil-Seed Industry: A Sustainable Approach. In Sustainable Food Waste Management. Springer, 2020, 177-190.
11. Vali SR, Ju YH, Kaimal TNB, Chern YT. A process for the preparation of food-grade rice bran wax and the determination of its composition. JAOCS, Journal of the American Oil Chemists' Society. 2005;82(1):57-64. https://doi.org/10.1007/s11746-005-1043-z
12. Wattanasiritham L, Theerakulkait C, Wickramasekara S, Maier CS, Stevens JF. Isolation and identification of antioxidant peptides from enzymatically hydrolyzed rice bran protein. Food Chemistry. 2016;192:156-162.
13. Wu X, Li F, Wu W. Effects of rice bran rancidity on the oxidation and structural characteristics of rice bran protein. LWT. 2020;120:108943.
14. Becker M, Staab D, Von Bergmann K. Long-term treatment of severe familial hypercholesterolemia in children. Effect of sitosterol and bezatibrate. Pediatrics. 1992; 89:138-

142.
15. Best MM, Duncan CH. Modification of abnormal serum lipid patterns in atherosclerosis by administration of sitosterol. Ann Intern Med. 1956; 45:614-622.
16. Sarfare S, Menon S, Shailajan S. Cornsilk as a bio-available source of beta-sitosterol: A pharmacokinetic study using HPTLC. Asian Journal of Plant Sciences. 2010; 9:44-50.
17. Rohini RM, Das AK. Determination of lupeol, beta-sitosterol, and quercetin from ethyl acetate extract of Rhizophora mucronata bark by HPTLC technique. Asian Journal of Pharmaceutical and Clinical Research. 2011; 4:103-105.
18. Attarde DL, Aurangabadkar VM, Belsare DP, Pal SC. Quantitative estimation of beta-sitosterol, lupeol, quercetin and quercetin glycosides from leaflets of Soymida febrifuga using HPTLC technique. Pak J. Pharm Sci. 2008; 21:316-319.
19. Shailajan S, Shah S, Syed N. HPTLC method development and validation of a secondary Metabolite – β-sitosterol from Ccaesalpinia bonduc (Linn.) Roxb. Emend. Dandy & Exell. Seed. International Journal of Pharma and Bio Sciences. 2010; 1:1-10.
20. Hareesh VK, Shashidhara S, Anitha S, Rajesh MS. Quantitative detection of reserpine in Rauwolfia serpentina using HPTLC. International Journal of pharmacy and Pharmaceutical sciences. 2010; 2:87-89.
21. Sundaram S, Perumal PC, Gopalakrishnan VK. Chromatographic and spectrophotometric analysis of bioactive compounds from Cayratia trifolia (L.) stem. Int J Pharm Pharm Sci 2018;8:56-64.
22. Mohammed FI, Al-Essa MK, Shafagoj YA, Afifi FU. Investigation of the direct effects of the alcoholic extract of Elaeagnus angustifolia L.(Elaeagnaceae) on dispersed intestinal smooth muscle cells of guinea pig. Sci Pharm 2006;74:21-30.
23. Katz GL, Shafroth PB. Biology, ecology and management of Elaeagnus angustifolia L.(Russian olive) in Western North America. Wetlands 2003;23:763-77.
24. Farzaei MH, Bahramsoltani R, Abbasabadi Z, Rahimi R. A comprehensive review on phytochemical and pharmacological aspects of Elaeagnus angustifolia L. J Pharm Pharmacol 2015;67:1467-80.
25. Tehranizadeh ZA, Baratian A, Hosseinzadeh H. Russian olive (Elaeagnus angustifolia) as a herbal healer. BioImpacts: BI 2016;6:155-67.
26. Trautwein EA, Demonty I. Phytosterols: Natural compounds with established and emerging health benefits. Oléagineux Corps Gras Lipides 2007;14:259-66.
27. Ahmed OH, Hamad MN, Jaafar NS. Phytochemical investigation of Chenopodium murale (Family: Chenopodiaceae) cultivated in Iraq, isolation and identification of scopoletin and gallic acid. Asian J Pharm Clin Res 2017;10:70-7.
28. Jamkhande PG, Barde SR, Patwekar SL and Tidke PS: Plant profile, phytochemistry and pharmacology of Cordia dichotoma (Indian cherry): A review. Asian Pacific Journal Tropical Biomedicine 2013; 3(12): 1009–1012.
29. Singh VH, Kaur S, Singh S, Bansal N: Pharmacological Review on Cordia dichotoma Frost. American Journal of PharmTech Res. 2017; 7(2): 85-96.
30. Vidyasagar G, Jadhav AG, Bendale AR and Sachin BN: Isolation of Cordia mucilage and its comparative evaluation as a binding agent with standard binder. Der Pharmacia Sinica 2011; 2(1):201-207.