RESEARCH ARTICLE

Isolation and Characterization of Novel Phenolic Compounds from the Aerial Parts of *Anaphalis triplinervis* (Sims) C.B. Clarke

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Received: 24th Jul, 2025; Revised: 6th Aug, 2025; Accepted: 3rd Sep, 2025; Available Online: 25th Sep, 2025

ABSTRACT
Medicinal plants are the best source of bioactive molecules and could serve as an appreciable source of many medicines. *Anaphalis triplinervis* (*A. triplinervis*), belonging to the family Asteraceae, is a perennial shrub that commonly grows in shady, moist habitats at elevations of around 2200 meters, and is generally found in India, Pakistan, and several other parts of the world. *A. triplinervis*, generally known as Bugla in Hindi and triple-veined pearly everlasting in English. The aerial parts of the plant are utilized to aid many diseases as well as serve as an important ethnomedicine. *A. triplinervis* is frequently used in bug bites, snake bites, as an anti-inflammatory, anti-asthmatic, anti-coughing, expectorant, sedative, and in convulsions. Isolation of ethyl acetate extract from *A. triplinervis* was chosen for the current investigation based on thin-layer chromatography and the first phytochemical screening. The ethyl acetate extract of the aerial parts of *A. triplinervis* was subjected to column chromatography following successive solvent extractions to isolate individual compounds. Every fraction of 20ml has been collected, and a total of 491 fractions were taken. Two phenolic compounds were isolated and initially confirmed by spraying a dilute ferric chloride solution on TLC plate. Further structure elucidation has been conducted by utilizing several spectrophotometric methods such as UV, IR, ^{1}H &^{13}C NMR, along with mass spectroscopy (MS). Based on spectral analysis, the compound was identified as Quercetin 3,7-di-O-rhamnoside, a flavonoid, along with caffeic acid, a phenolic acid.

Keywords: *Anaphalis triplinervis*, extraction, isolation, characterization
How to cite this article: Ankush Sundriyal, Aanchal Loshali, Bhuwan Chandra Joshi, Minky Mukhija, Yogita Dobhal. Isolation and Characterization of Novel Phenolic Compounds from the Aerial Parts of *Anaphalis triplinervis* (Sims) C.B. Clark. International Journal of Drug Delivery Technology. 2025;15(3):1017-21. doi: 10.25258/ijddt.15.3.16
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Each year, thousands of novel organic compounds and molecules are reported by Phytochemists. One important method for finding and creating new medications is the pharmacological testing, chemical modification, derivatization, and study of these natural compounds [1]. Recently, structural characterization, isolation, and combinatorial preparations have given plant products a renewed interest as potential sources of novel pharmaceuticals[2]. Plant-based natural products contribute significantly to drug development and are a rich source of molecules with remarkable chemical and functional variety[3]. Isolating the secondary metabolites from natural resources has been the subject of extensive investigation worldwide[4]. Genus *Anaphalis* contains small herbs and shrubs, found in Asia, Europe, and America[5]. There are approximately 34 species known to exist in India.

It is the largest genus in the Asteraceae family[6]. Many species of plants in this genus are utilised as a source of traditional medicines and new, uncommon phenolic compounds[7]. Numerous species of *Anaphalis* have long been used as sedatives, expectorants, anti-epileptics, anti-phlogistics, anti-asthmatics, and against cough[8]. *A. triplinervis* is an erect perennial herb having weak branching stem with a woody or cottony appearance[9,10]. Many ethnopharmacological uses of the *A. triplinervis* are also documented, including diuretics[11-13], wound healing[14,15], cough remedies[13], anti-emetics[16], anti-pyretics[17], and issues relating to menstruation[18].

A. triplinervis contains various essential oils such as 1, 10-epoxyfuranoeremophilane, valencene, α-humulene, epi-α-cadinol, germacrene, farnesene, and β-vetivone. Other important components include β-caryophyllene, (Z)-β-ocimene, β-elemene, p-cymene, caryophyllene oxide, and humulene epoxide II[11,12].

However, a significant research gap exists since flavonoids and phenolic compounds from A. triplinervis have not yet been identified or isolated.

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The present research aimed to isolate and identify phytochemicals from the aerial parts of *A. triplinervis*. For the first time, two compounds, caffeic acid and the flavonoid quercetin 3,7-di-O-rhamnoside, were successfully isolated from this plant. Phenolic compounds, which represent a key group of phytoconstituents, were separated using Thin Layer Chromatography (TLC) and column chromatography. The structural characterizations of the isolated compounds were carried out using various spectroscopic techniques, including IR, ^{1}H & ^{13}C NMR, and mass spectrometry (MS).

MATERIALS AND METHODS

Collection and Authentication

Aerial plant parts of *A. triplinervis* were collected from Dhanolti area of Uttarakhand India. The specimen was confirmed by Dr. S. K. Singh, Scientist-E/Head of Office, Botanical Survey of India, with Accession number 1193, and the plant was shade-dried prior it was delivered to the Botanical Survey of India's Northen Regional Centre, Dehradun (BSD student herbarium).

Chemicals and Instruments

All reagents and chemicals utilised in the current investigation were of analytical grade and gathered from credible suppliers (SRL, Merck, and Ranbaxy). HPTLC aluminium sheets (20x20cm), Merck KGaA, Silica gel 60 F254, Germany, have been used for developing the chromatogram. Structural elucidation and chemical identification were carried out using NMR, UV, IR as well as MS.

Extraction

The aerial parts of *A. triplinervis* have been cleaned thoroughly with water and further shade-dried. After the complete drying process plant material was coarsely powdered utilizing a mechanical blender before extraction and stored in a closed container for further use.

Successive Solvent Extraction

For the extraction of *A. triplinervis* aerial parts, a Soxhlet extraction apparatus was employed. The upper chamber of the apparatus contained a permeable thimble containing 500 g of powdered aerial parts material. The round-bottom flask was filled with two 2.5 litres of extracting solvent. A thermostat-controlled heating mantle was used to warm the flask. Successive extraction was carried out using solvents in increasing order of polarity namely petroleum ether, chloroform, ethyl acetate, ethanol, and water. Temperature was regulated according to the solvent's boiling points. After complete extraction, the extract has been concentrated in a rotary evaporator under decreased pressure. All extracts were refrigerated until further usage.

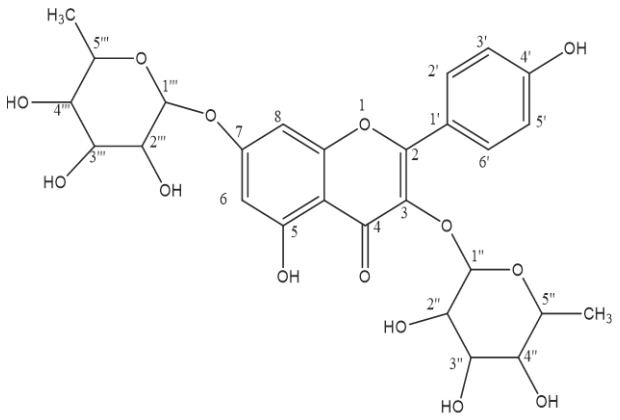

Figure 1: Quercetin 3,7-di-O-rhamnoside

Phytochemical Analysis

Preliminary phytochemical analysis of all the extracts has been conducted in order to identify active secondary metabolites or additional constituents, including tannins, alkaloids, flavonoids, terpenoids, steroids, carbohydrates, proteins, and saponins[19]. Dried extracts obtained have been reconstituted in methanol or other solvents as required or each extract was subjected to a preliminary phytochemical examination.

Thin Layer Chromatography (TLC)

All the extracts obtained by successive solvent extraction were subjected to TLC technique to find out the presence of several phytochemicals[20]. Silica gel G (Merck, 0.25mm thickness) plates have been utilized for better resolution in TLC. The various solvent systems were tried for each extract to find an appropriate solvent system. The solvent system were selected; petroleum ether extract (toluene: ethylacetate (4.5: 0.5 v/v)), chloroform extract give best resolution in (toluene: methanol (4.5: 0.5 v/v)), solvent system selected for ethylacetate extract (toluene: chloroform: methanol (4:4:1, v/v/v)), ethanolic extract (ethylacetate: glacial acetic acid: formic acid: water (100:11:11:26 v/v/v/v)) and water extract was chromatographed with n- butanol: acetic acid: methanol: water (4.5: 1.5:0.5: 0.5 v/v/v/v) solvent system. Right after formation of chromatograms in solvents, plates were dried and subjected to a dilution of $FeCl_3$ and an anisaldehyde sulphuric acid detecting reagent in order to identify flavonoids and phenolics. Visualization of TLC plate was also carried out under short and long-wavelength UV lights.

Isolation using Column Chromatography

The ethyl acetate extract of *A. triplinervis* aerial parts was further taken for isolation to column chromatography, as the extract shows significant amount of phenolic, flavonoids in a preliminary study, as compared to other extracts. For isolation, the column (5x100 cm) has been filled with silica gel (60-120 mesh size). Column has been eluted using a gradient of toluene and ethyl acetate. Each 20 ml portion was collected, concentrated, and allowed to crystallize. A total of 435 fractions were gathered. Fractions having same R_f values were grouped together. Isolated chemical has been purified by successive recrystallization and solvent washing.

The fractions 361- 373 on elution with toluene: ethyl acetate (70: 30) produced light-yellow residue (96 mg) that presented single spot on TLC plate. As repeated recrystallization with methanol it formed colourless needle-shaped crystalline compound along with its homogeneity has been validated by TLC researches as well as regarded as compound ATF1 (Yield 37 mg,). Such compound was subjected to physical and spectral researches for ensuring purity and characterization. Another compound was isolated from fraction 416-432 as light brown colour powder (72mg) on elution with toluene: ethyl acetate (5:5). Further recrystallized of isolated compound was done with

methanol and single spot confirm by TLC analysis and designated as ATF2 (35 mg) and subjected for spectroscopy.

Compound Characterizations

NMR, UV, IR as well as mass spectra were used to characterise molecule. Digital melting point equipment has been utilized to measure melting point of the isolated compounds. Using a Shimadzu UV-1800, the isolated constituent's UV absorbance in methanol was measured over a 200–800 nm scanning range. To achieve the necessary concentration, the chemical was dissolved in methanol, and the resulting spectra were noted. Perkin Elmer spectra Version 10.03.05 was used to determine the isolated constituents' infrared absorption spectra, and the absorption peaks were recorded as wave numbers (cm-1). A Bruker DPX-400 spectrometer for both ^{1}H (500 MHz) and ^{13}C (400 MHz) NMR spectra after the materials were dissolved in DMSO.

RESULTS AND DISCUSSION

Successive solvent extraction of the aerial parts of *A. triplinervis* revealed that the highest extract yield (3.85% w/w) was obtained with ethyl acetate. Table 1 displays the various extracts' colour, consistency, odour, and yield.

The qualitative chemical tests revealed the presence of numerous phytocomponents is various extracts. Carbohydrates, tannins, saponins, flavonoids, phenolics, steroids, and triterpenoids were present in various extracts of *A. triplinervis* aerial parts (Table 2). Qualitative tests indicated that the ethyl acetate extract could be a significant source of phytochemicals with therapeutic potential, as it gave positive results in the Shinoda and dilute $FeCl_3$ tests.

Further, different extracts have been subjected to TLC analysis to check the number of constituents present in a specific extract. Petroleum ether extract exhibited availability of seven spots; chloroform extract showed eight spots. However highest number of spots is shown by ethyl acetate extract, in a mobile phase Toluene: Chloroform: methanol (4:4:1v/v/v) with nine spots respectively. Ethanolic extract give five spots, and aqueous extracts gives three spots (Table 3).

On the basis of outcomes of TLC, ethyl acetate extract has been determined to bear highest number of phytoconstituents. Phytochemical screening of ethyl acetate extract also showed strong positive tests for phenolics and flavonoids. Moreover, the percentage yield of this extract was the highest among all extracts. As a result, two compounds were separated and purified from the ethyl acetate extract, which was chosen for further compound isolation using column chromatography.

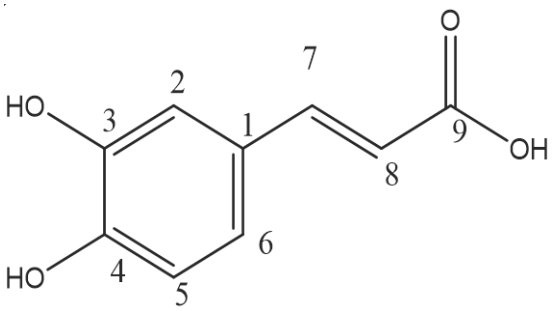

Figure 2: Caffeic acid

Table 1: Percentage extractive and characteristic of extracts

Plant Extract	Colour	Consistency	Odour	% (W/W)
Petroleum Ether Extract	Yellowish	Sticky semisolid	Characteristic, disagreeable	1.96
Chloroform Extract	Dark Green	Solid	Unpleasant	2.76
Ethyl Acetate Extract	Brownish green	Semisolid	Characteristic, agreeable	3.85
Ethanolic Extract	Dark Brown	Semisolid	Faint	3.70
Aqueous Extract	Brownish Black	Solid mass	Faint	3.56

Compound ATF 1 has been isolated as pale yellow colored crystals, 37 mg (0.74% w/w yield); Rf 0.34 (Toluene: ethyl acetate 70: 30); m.p. 268-271°C; UV λmax (methanol) was 316nm; the compound gives a sharp test with $FeCl_3$, and a positive Shinoda test for flavonoid.

IR (KBr) spectrum presented stretching for 3430 cm^{-1} Broad O–H stretching indicate multiple phenolic & sugar–OH groups (broad due to H-bonding), 1654 cm^{-1}C=O stretching Conjugated ketone in chromone ring, 1606 cm^{-1}C=C stretching (aromatic) Aromatic ring vibrations from chromone and phenyl rings, 1249 cm^{-1}C–O stretching Phenolic C–O and C–O–C glycosidic linkages, 1067 cm^{-1}C–O–C and C–OH (sugar region)Ether and sugar ring C–O vibrations.

^{1}H NMR showed signals which indicates, ^{1}H NMR (500MHz, DMSO) δC: 1.25 (d, H, J = 6.2 Hz, CH_3), 1.29 (d, H, J = 6.4 Hz, CH_3), 2.60-2.01 (s, 24H, 8xCH_3 of $OCOCH_3$), "3.54 (m, 1H, 5'"-H), 3.54 (m,1H,5"-H), 3.64 (t, **J = 9.2 Hz**, 1H, 3"-H), 3.65 (t, J = 9.4 Hz, 1H,4"-H), 3.65 (t, **J = 9.0 Hz**,1H, 4'"-H), 3.69 (t, **J = 9.1 Hz**,1H, 3'"-H), 3.72 (t, **J = 9.0 Hz**, 1H, 2"-H), 3.72 (t, J = 9.0 Hz, 1H,2'"-H), 5.04 (d, **J = 7.4 Hz**, 1H,1"-H), 5.04 (d, **J = 7.2 Hz**, 1H, 1'"-H), 7.14 (d, **J = 8.6 Hz**, 1H, 6'-H), 7.26 (d, **J = 8.8 Hz,**1H, 2'-H), 7.40 (d, **J = 8.5 Hz**,1H, 5'-H), 7.44 (d, **J = 8.7 Hz**,1H,3'-H), 7.60 (s, 1H, H-8), 7.71 (s, 1H, H-6).

Structure has been further confirmed by investigation of ^{13}C NMR (500 MHz, DMSO):δ 44.86 (C-2), 43.93 (C-3), 52.07 (C-4), 175.59 (C-5), 133.57 (C-6), 170.68 (C-7), 130.35 (C-8), 166.63 (C-9), 149.22 (C-10), 147.97 (C-1'), 126.73 (C-2'), 127.89 (C-3'), 169.94 (C-4'), 128.75 (C-5'), 125.61 (C-6'), 44.86 (C-1"), 40.80 (C-2"), 39.29 (C-3"), 32.30 (C-4"), 31.82 (C-5"), 43.93 (C-1'"), 40.81 (C-2'"), 32.30 (C-3'"), 32.30 (C-4'"), 32.82 (C-5'")", 14.0 (2xCH_3), 30.57-21.58 (CH_3 of 8x$OCOCH_3$), 175.59-166.63 (CO of 8x$OCOCH_3$),

HR-ESI-MS (positive): m/z 637.1573 $[M+K]^+$

Hence based on above spectral data, compound ATF1 is characterized type of flavonoid glycoside, termed as Quercetin 3,7-di-O-rhamnoside (Figure 1), having a molecular formula $C_{27}H_{30}O_{15}$.

As best of our literature review, for the first time Quercetin 3,7-di-O-rhamnoside was isolated and characterized from a

Table 2: Preliminary phytochemical screening of various extracts of *Anaphalis triplinervis* aerial parts

Phytochemical Test	Pet. Ether Extract	Chloroform Extract	Ethyl Acetate Extract	Ethanolic Extract	Aqueous Extract
Carbohydrates	-	-	-	+	+
Glycosides	-	-	+	+	+
Saponin	-	-	-	-	+
Tannins	-	+	++	-	-
Flavonoids	-	-	++	-	-
Phenolics	-	+	++	+	-
Amino acid	-	-	-	+	+
Steroids	+	-	-	-	-
Terpenoids	++	-	-	-	-
Proteins	-	-	-	-	+
Fatty acids	+	-	-	-	-
Alkaloids	-	-	-	-	-

Table 3: Rf values of phytochemicals in various extracts of *Anaphalis triplinervis* aerial parts

Extract	Solvent system	No. of spots	Rf values
Pet. Ether Extract	Toluene: Ethyl acetate (4.5: 0.5 v/v).	7	0.33,0.35,0.4,0.45,0.56,0.63,0.77
Chloroform Extract	Toluene: Methanol (4.5: 0.5 v/v	8	0.13, 0.16, 0.23, 0.27, 0.37, 0.38, 0.47, 0.61
Ethyl acetate extract	Toluene: Chloroform: Methanol (4:4:1)	9	0.07, 0.22, 0.36, 0.41, 0.45, 0.47, 0.58, 0.64, 0.67
Ethanolic extract	Ethyl acetate: Glacial acetic acid: Formic acid: Water (100:11:11:26)	5	0.22, 0.39, 0.59, 0.74, 0.87
Aqueous extract	n- butanol: Acetic acid: Methanol: Water (4.5: 1.5:0.5: 0.5)	3	0.23, 0.37, 0.43

A. triplinervis plant. Although quercetin glycosides have been the subject of much research, rhamnose-conjugated derivatives, especially dirhamnoside, have not yet been reported in phytochemical literature of plant. In addition to expanding the known range of naturally occurring quercetin glycosides, this new finding will creates additional opportunities to investigate their possible pharmacological characteristics.

Compound ATF 2 was obtained as light brownish colored crystals, 35 mg (0.70% w/w yield); Rf 0.48 (toluene: ethyl acetate 50:50); m.p. 211–214°C; compound gives positive test for dilute $FeCl_3$ solution. UV λmax (methanol): 267 and 316nm. IR (KBr) displayed stretching at 3433 cm^{-1} for phenolic and carboxylic (broad due to H- Bonding), 1646cm^{-1}, C=O stretch for carboxylic acid carbonyl group, 1526 cm^{-1} Aromatic C=C stretch Conjugated benzene ring vibrations, 1619 cm^{-1} Alkene C=C stretch C=C from the propenoic side chain, 1217 cm^{-1} C–O stretch (phenolic) Phenolic hydroxyl groups, 972 cm^{-1} =C–H out-of-plane bending Trans-alkene (=CH) bending vibration. Compound ATF 2 show 1 H NMR signals at ^{1}H "NMR (500 MHz, DMSO-d_6) δ: 6.20 (d, 1H, J = 16.0 Hz, H-7),6.78 (d, 1H, J = 8.0 Hz, H-5'), 6.85 (dd, 1H, J = 8.0, 2.0 Hz, H-6'), 7.01 (d, 1H, J = 2.0 Hz, H-2'), 7.45 (d, 1H, J = 16.0 Hz, H-8), 9.15 (br s, 1H, –OH), 9.65 (br s, 1H, –OH),10.90 (br s, 1H, COOH). 13C NMR displayed signals in ^{13}C NMR (500 MHz, DMSO-d_6) δ:115.2 (C-2'), 116.3 (C-5'), 121.4 (C-6'), 123.7 (C-1'), 127.6 (C-3'), 145.9 (C-4'), 114.5 (C-7), 146.8 (C-8), 146.5 (C-3), 127.8 (C-1"), 168.3 (C-9, –COOH).

Mass spectra show peak $[M - H]^-$ = m/z 179.0 — Base peak, very strong signal, Fragments m/z 135 – loss of CO_2, m/z 85 – further fragmentation. Based on the above spectral studies, compound ATF2 was characterized as caffeic acid (figure 2), which is well known for its pharmacological potential.

CONCLUSION

Both phenolic compounds herein described were isolated first time from *A. triplinervis* aerial parts. Quercetin-3,7-diriboside, is a flavonoid glycoside which is first time isolated from plant, best of our information from literature review. This study reported ribose-conjugated quercetin first time in phytochemical literature. Caffeic acid is another compound which is reported in this study, it is a phenolic acid having numerous pharmacological activities as per previous reports, and so present study creates ample opportunities for pharmacological evaluation of *A. triplinervis*. In the future, various *in vitro* and *in vivo* studies can be conducted on the isolated compound to determine its safety and efficacy.

Acknowledgements

The authors acknowledge the School of Pharmaceutical Sciences and Technology for providing all necessary assistance.

REFERENCES

1. Egbuna C, Kumar S, Ifemeje JC, Ezzat SM, Kaliyaperumal S, editors. Phytochemicals as lead compounds for new drug discovery. Elsevier. 2019;7.
2. Brusotti G, Cesari I, Dentamaro A, Caccialanza G, Massolini G. Isolation and characterization of bioactive

compounds from plant resources: the role of analysis in the ethnopharmacological approach. J. Pharm. Biomed. Anal. 2014;87:218-28.
3. Chaachouay N, Zidane L. Plant-derived natural products: a source for drug discovery and development. DDC.2024;3(1):184-207.
4. Patil RH, Patil MP, Maheshwari VL. Extraction and Isolation of Secondary Metabolites from Apocynaceae Plants. In Apocynaceae Plants: Ethnobotany, Phytochemistry, Bioactivity and Biotechnological Advances. Springer. 2023:37-50.
5. Xu XM, Xu H, Yang Z, Wei Z, Gu JY, Liu DH, Liu QR, Zhu SX. Phylogeny, biogeography, and character evolution of Anaphalis (Gnaphalieae, Asteraceae). Front. Plant Sci. 2024;15:1336229.
6. Murugan P, Murugan C, Karthigeyan K. Typification of twelve names in the genus Anaphalis DC.(Asteraceae). Adansonia. 2021;43(17):197-204.
7. Murai Y, Yangzom R, Wangmo C, Dorji R. Phenolic compounds from three alpine Anaphalis species in Japan and Bhutan. Bull. Natl. Mus. Nat. Sci., Ser. B. 2021;47:109-14.
8. Rawat K, Prasad K, Bisht G. Phytochemical analysis and antioxidant activity essential oil of Anaphalis contorta from Uttrakhand Himalayas. J Anal Pharm Res. 2017;6(2):00172.
9. Chaurasia OP, and Singh B. Cold desert flora (I-V) Field Research Laboratory. Leh- Ladakh. DESIDOC, Delhi. 1996-2001; 34
10. Kumar A, Choudhary A, Kaur H. Diversity of wild medicinal flora in Lahaul valley of Himachal Pradesh. IJCMAS. 2020; 9: 48-62.
11. Malla B, Gauchan DP, Chhetri RB. An ethnobotanical study of medicinal plants used by ethnic people in Parbat district of western Nepal. J. Ethnopharmacol.2015; 165: 103-17.
12. Sharma RK, Kaur H, Singh M, Kumar M, Sharma R, Shah GC, Sharma P. Chemical composition and antimicrobial properties of essential oil *Anaphalis triplinervis* from Western Himalaya. Chem. Nat. Compd. 2019; 55(4): 751-3.
13. Bisht VK, Purohit V. Medicinal and aromatic plants diversity of Asteraceae in Uttarakhand. Nat Sci. 2010; 8(3): 121-128.
14. Balami NP. Ethnomedicinal uses of plants among the Newar community of Pharping village of Kathmandu district, Nepal. Tribhuvan University Journal. 2004; 24(1): 13-9.
15. Hailu T. Phytopharmaceutical studies of some selected medicinal plants locally used in the treatment of skin disorders (Doctoral dissertation, Addis Ababa University). 2004.
16. Ahmed S, Hasan MM, Ahmed SW. Natural antiemetics: an overview. Pak. J. Pharm. Sci. 2014; 27.
17. Gorsi MS, Miraj S. Ethenomedicinal survey of plants of Khanabad village and its allied areas, District Gilgit. Asian J. Plant Sci. 2002.
18. Gewali MB, Awale S. Aspects of traditional medicine in Nepal. Japan: Institute of Natural Medicine University of Toyama. 2008;140-142.
19. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media; 1998.
20. Wagner H, Bladt S. Plant drug analysis: a thin layer chromatography atlas. Springer Science & Business Media; 1996.