Research Article

Isolation of Nepetin (6-Methoxyluteolin) from *Artemisia vulgaris* and Spectroscopic Characterization: A Bioactive Flavonoid

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Abstract | *Artemisia vulgaris*, a medicinal plant with various pharmacological properties, has a longstanding history of global utilization. The biochemical components substantially impact the economy and pharmacological sector, providing various advantages. Nepetin, a flavonoid found in Artemisia plants, has been shown to possess various biological effects including anti-oxidant, anti-inflammatory, anti-cancer, and anti-apoptotic properties. In this study, nepetin as yellowish powder was isolated from *A. vulgaris* extract through column chromatography. Structure of the compound was elucidated through various spectroscopic techniques such as Mass and NMR (1D and 2D).

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Introduction

Artemisia vulgaris (*A. vulgaris*), a significant medical plant species in the genus *Artemisia*, is known for its volatile essential oils. The biological and chemical variety of the genus *Artemisia,* along with the discovery and isolation of the potential malarial treatment compound artemisinin, have drawn the great attention (Abiri *et al.*, 2018). Herbal remedies from *A. vulgaris* have been used globally for treating various diseases, including malaria, inflammation, infection prevention, hepatoprotective, immunomodulatory, hypertensive, cancer, and anti-oxidant (Ekiert *et al.*, 2020). These remedies are primarily based on organic acids, acetylenes, coumarins, sesquiterpene lactones, phenolic acids, flavonoids, and other secondary metabolites (Siwan *et al.*, 2022). There has been a considerable increase in studies relating to its phytochemistry, anatomy, and morphology to better understand the formation and accumulation of medicinal chemicals in *A. vulgaris*. The medicinal potential of bioactive substances derived from *A. vulgaris* has been recently supported by pharmacological and phytochemical studies (Sharma and Adhikari, 2023; Singh *et al.*, 2023; Siwan *et al.*, 2022). These results add to the growing body of information that should be considered when trying to synthesize more affordable alternatives to existing anti-malarial medications through the discovery and isolation of new chemicals.

Nepetin, referred to as 6-Methoxyluteolin or Eupafolin, is a naturally occurring flavonoid found in several plant species, including the *Artemisia* herb (Zhang *et al.*, 2017). Nepetin has demonstrated a range of biological effects such as anti-oxidant, anti-inflammatory, anti-cancer and anti-apoptotic properties (Liu *et al.*, 2015; Maas *et al.*, 2011). It demonstrated a strong capacity to inhibit bone resorption and osteoclast differentiation *in vitro*, while also providing protection to mice against Ti particle–induced calvarial osteolysis through decreasing both the activity and quantity of osteoclasts (Chu *et al.*, 2020). Nepetin minimizes the expression of virulence factors by specifically targeting ClpP in the context of multi-drug-resistant *S. aureus*-induced pneumonia infection (Jing *et al.*, 2022). Yoon *et al.* (2022) discovered that nepetin, a multi-targeting inhibitor of Protein Tyrosine Phosphatases (PTPs), decreases the catalytic activity of PTPs in vitro and enhances glucose absorption in mature 3T3-L1 adipocytes, indicating its potential as a therapeutic candidate for type 2 diabetes treatment (Yoon *et al.*, 2022). Herein, we reported the detail procedure of isolation of nepetin (6-Methoxyluteolin) from *A. vulgaris* and its structure elucidation through various spectroscopic techniques.

Materials and Methods

General experimental

2.75 kg of air-drying plant material is ground or chopped into small fragments to form a homogeneous powder and subsequently macerated at a ratio of 1 g to 10 mL in methanol. The following flowchart delineates the extraction process (Figure 1).

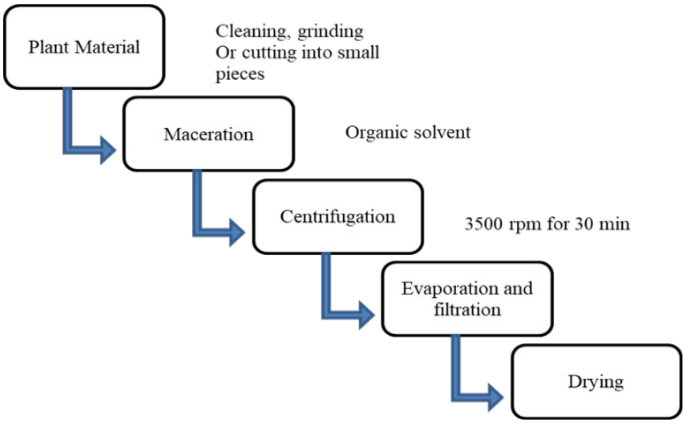

Figure 1: *Extraction process.*

The crude extract was filtered using a silica gel and Merck alumina column, while semi-pure fractions underwent chromatography on an LH-20 Sephadex packed column (Figure 2). Thin-layer chromatography was employed to identify and quantify chemicals. Silica gel-coated TLC plates were employed to assess the purity of chemicals in extracts or their pure forms. The compounds were examined under UV light for fluorescence quenching and fluorescent staining, with their spots seen using ceric (IV) sulfate for flavonoid testing.

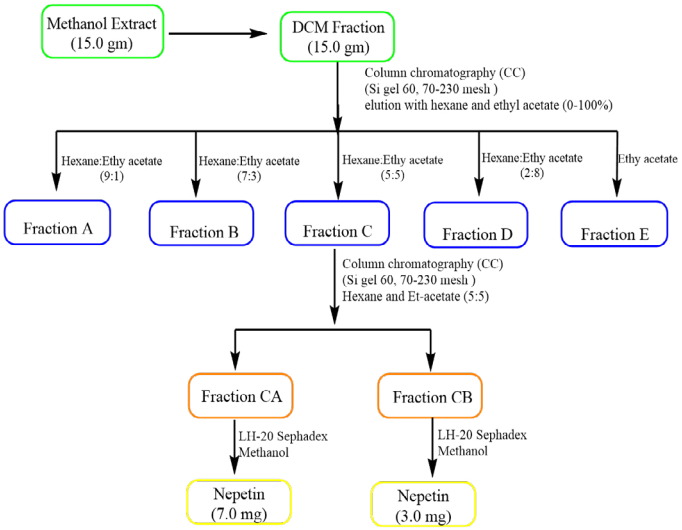

Figure 2: *Isolation of nepetin from A. vulgaris.*

Spectroscopic techniques

The study employed a mass spectrometer to get EI-MS spectra, utilizing the Jeol MS pathway in conjunction with TSS 2000. Bruker Avance instruments were employed to acquire ^{1}H-NMR and ^{13}C-NMR spectra at different frequencies. The multiplicity of carbon signals was assessed using DEPT 90° and 135° tests, while the impacts of homo-nuclear ^{1}H-^{1}H connection were determined by the COSY 45° experiment. HSQC and HMBC studies were performed to ascertain one-bond and two- and three-bond ^{1}H-^{13}C connectivity.

Results and Discussion

Homogenized plant material of *A. vulgaris* was macerated with 100% methanol for three days. After dissolving the extract in distilled water, it underwent fractionation using hexanes. 15.0 gm of DCM fraction has been obtained by extracting the watery layer with CH_2Cl_2, and removed the remaining 20 gm of watery layer with ethyl acetate. LH-20 sephadex and silica gel columns were used for chromatography of the ethyl acetate fractions and DCM, respectively. The yellow solid known as nepetin (6-methylluteolin)

was obtained by employing column chromatography of the DCM fraction with a mobile phase consisting of hexane: Ethylacetate (1:1) and methanol (1:2) ratio. After spurting with cerric sulphate spray, it was observed as a yellow pustule on a silica gel TLC plate.

Nepetin's EI-MS spectrum showed the base peak and a [M+H] at m/z 316 (Figure 3). A breakdown of nepetin produced the important fragment ions at m/z 135, 167, 301, and 316. The isolated component was determined to be a flavonoid based on the fragment sequence.

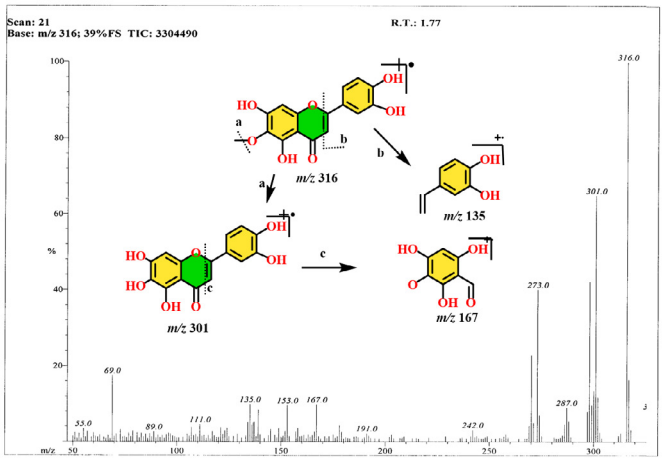

Figure 3: *Characteristics EI-MS fragmentation of nepetin.*

The ^{1}H-NMR spectrum of nepetin, as presented in Table 1, exhibited a resonance at δ 3.87, which was assigned to the protons of the methoxy group linked to C-6. The signal observed at δ 6.54 s was attributed to H-8 and H-3, respectively. Three downfield signals were observed at 7.38dd (*J*2', 6'=1.8 Hz; *J*5', 6' =7.8,), 6.88d (*J*6', 5'=7.8 Hz), and δ 7.36d (*J*6', 2'=1.8 Hz) which were assigned to protons H-6', H-5', and H-2', respectively. The comprehensive analysis of the DEPT and BB ^{13}C-NMR spectra (Table 1) indicated the presence of resonance for sixteen distinct carbon atoms, comprising ten quaternary carbon centers, five methine units, and one methyl group.

In order to conform the structure of nepetin and determine the location of the methoxy group, 2D NMR (NOESY, COSY, HMBC, and HSQC) spectroscopy was utilized (Figure 4). Figure 4 clearly demonstrated the location of the methoxy group in the molecule as the HMBC interaction between protons and carbons resonated at δ 132.8 (C-6) and δ 3.87. The isolated compound correlates with previously reported compound nepetin in all spectroscopic and physical measurements (Shim *et al.*, 2012).

Table 1: *^{1}H- and ^{13}C-NMR chemical shift values of nepetin.*

Carbon No.	δ_C	δ_H (*J*, Hz)
2	165.7	-
3	103.1	6.54 s
4	183.0	-
5	152.7	-
6	132.8	-
7	159.3	-
8	95.0	6.54 s
9	155.9	-
10	106.0	-
1'	124.0	-
2'	114.8	7.36 d (1.8)
3'	148.0	-
4'	151.1	-
5'	116.1	6.88 d (7.8)
6'	120.2	7.38 dd (7.8, 1.8)
6-OMe	61.1	3.87 s

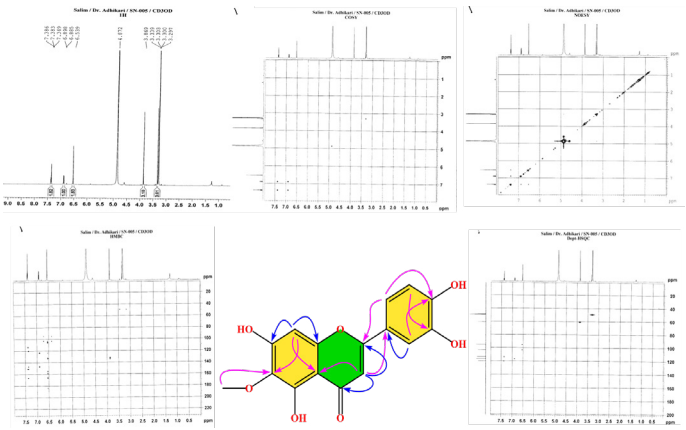

Figure 4: *1D and 2D NMR spectra and characteristic HMBC correlations of nepetin.*

Conclusion and Recommendations

The results reveal that extracts from *A. vulgaris* are a rich source of nepetin, a bioactive flavonoid. Nepetin has exhibited several biological effects, including antioxidant, anti-inflammatory, anti-cancer, and anti-apoptotic activities. Consequently, the extract of *A. vulgaris* may serve as a foundational component for the production of herbal products to treat diseases.

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Novelty Statement

Nepetin, a flavonoid found in Artemisia plants, has

been isolated and its structure elucidated using spectroscopic techniques, showing potential in various biological applications.

Author's Contribution

Salim Saifullah: Conceptualization, methodology, data curation, data interpretation, data visualization.
Achyut Adhikari: Conceptualization, supervision, plant material collection, reviewing and editing original draft and revision and writing first draft and writing revised drafts.

Conflict of interest
The authors have declared no conflict of interest.

References

Abiri, R., Silva, A.L.M., de Mesquita, L.S.S., de Mesquita, J.W.C., Atabaki, N., de Almeida Jr, E.B., Shaharuddin, N.A. and Malik, S., 2018. Towards a better understanding of *Artemisia vulgaris*: Botany, phytochemistry, pharmacological and biotechnological potential. Food Res. Int., 109: 403-415. https://doi.org/10.1016/j.foodres.2018.03.072

Chu, B., Chen, S., Zheng, X., Ye, J., Cheng, X., Zhang, L., Guo, D., Wang, P., Hong, D., and Hong, Z., 2020. Nepetin inhibits osteoclastogenesis by inhibiting RANKL-induced activation of NF-κB and MAPK signalling pathway, and autophagy. J. Cell. Mol. Med., 24(24): 14366-14380. https://doi.org/10.1111/jcmm.16055

Ekiert, H., Pajor, J., Klin, P., Rzepiela, A., Ślesak, H. and Szopa, A., 2020. Significance of *Artemisia vulgaris* L. (Common Mugwort) in the history of medicine and its possible contemporary applications substantiated by phytochemical and pharmacological studies. Molecules, 25(19): 4415. https://doi.org/10.3390/molecules25194415

Jing, S., Ren, X., Wang, L., Kong, X., Wang, X., Chang, X., Guo, X., Shi, Y., Guan, J., Wang, T., Wang, B., Song, W. and Zhao, Y., 2022. Nepetin reduces virulence factors expression by targeting ClpP against MRSA-induced pneumonia infection. Virulence, 13(1): 578-588. https://doi.org/10.1080/21505594.2022.2051313

Liu, K., Park, C., Chen, H., Hwang, J., Thimmegowda, N., Bae, E.Y., Lee, K.W., Kim, H.G., Liu, H. and Soung, N.K., 2015. Eupafolin suppresses prostate cancer by targeting phosphatidylinositol 3-kinase-mediated Akt signaling. Mol. Carcinogen., 54(9): 751-760. https://doi.org/10.1002/mc.22139

Maas, M., Deters, A.M. and Hensel, A., 2011. Anti-inflammatory activity of *Eupatorium perfoliatum* L. extracts, eupafolin, and dimeric guaianolide via iNOS inhibitory activity and modulation of inflammation-related cytokines and chemokines. J. Ethnopharmacol., 137(1): 371-381. https://doi.org/10.1016/j.jep.2011.05.040

Sharma, K.R. and Adhikari, S., 2023. Phytochemical analysis and biological activities of *Artemisia vulgaris* grown in different altitudes of Nepal. Int. J. Food Prop., 26(1): 414-427. https://doi.org/10.1080/10942912.2023.2166954

Shim, S.Y., Park, J.R. and Byun, D.S., 2012. 6-Methoxyluteolin from *Chrysanthemum zawadskii* var. latilobum suppresses histamine release and calcium influx via down-regulation of $ Fc {\varepsilon} RI $${\alpha} $ chain expression. J. Microbiol. Biotechnol., 22(5): 622-627. https://doi.org/10.4014/jmb.1111.11060

Singh, N.B., Devi, M.L., Biona, T., Sharma, N., Das, S., Chakravorty, J., Mukherjee, P.K. and Rajashekar, Y., 2023. Phytochemical composition and antimicrobial activity of essential oil from the leaves of *Artemisia vulgaris* L. Molecules, 28(5): 2279. https://doi.org/10.3390/molecules28052279

Siwan, D., Nandave, D. and Nandave, M., 2022. *Artemisia vulgaris* Linn: An updated review on its multiple biological activities. Future J. Pharma. Sci., 8(1): 47. https://doi.org/10.1186/s43094-022-00436-2

Yoon, S.Y., Ahn, D., Kim, J.K., Seo, S.O. and Chung, S.J., 2022. Nepetin acts as a multi-targeting inhibitor of protein tyrosine phosphatases relevant to insulin resistance. Chem. Biodiv., 19(1): e202100600. https://doi.org/10.1002/cbdv.202100600

Zhang, H., Chen, M.K., Li, K., Hu, C., Lu, M.H. and Situ, J., 2017. Eupafolin nanoparticle improves acute renal injury induced by LPS through inhibiting ROS and inflammation. Biomed. Pharmacother., 85: 704-711. https://doi.org/10.1016/j.biopha.2016.11.083