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Chemical composition and biological activities of essential oil from *Grewia bulot* leaves

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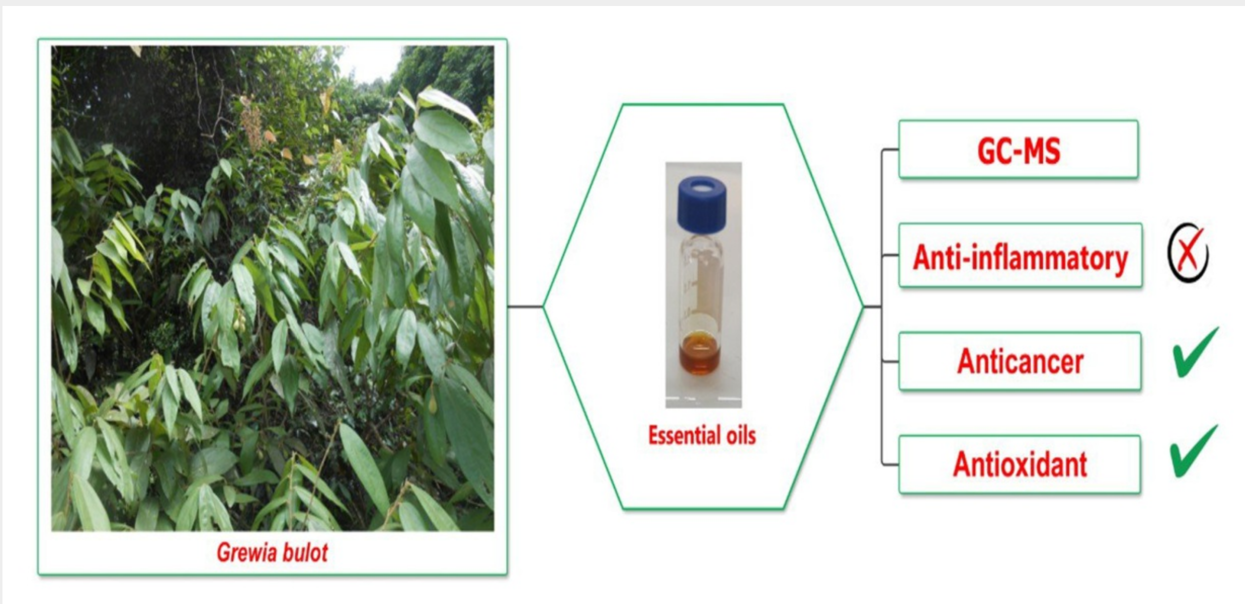
ABSTRACT

This study focused on the chemical composition and biological activities of the essential oil derived from *Grewia bulot*, a plant species known for its medicinal properties. The analysis of *Grewia bulot* essential oil revealed the presence of 78 constituents. The major compounds were *α*-cadinol (13.5%), 1,8-cineole (12.7%), 1,10-di-epi-cubenol (9.8%), epi-*α*-cadinol (6.7%), (*E*,*E*)-*α*-farnesene (5.9%), (*E*)-citral (4.0%), selin-11-en-4-*α*-ol (4.0%), citronellol isobutanoate (3.9%), and geranic acid (3.7%). The essential oil exhibited promising antioxidant potential with an IC_{50} value of 452.65 ± 28.40 µg/mL in DPPH model. This oil did not show NO production inhibitory effect in RAW264.7 cells. In addition, the essential oil exhibited significant cytotoxicity against KB, Hep-G2, MCF-7, and SK-LU-1 cancer cell lines, with IC_{50} values ranging from 44.04 ± 1.47 to 74.20 ± 3.71 μg/mL.

Graphical Abstract



KEYWORDS

Grewia bulot; essential oil; GC-ms; antioxidant; anti-inflammatory; anticancer

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1. Introduction

Grewia is a genus of shrubs or small trees belonging to the Malvaceae family (Kumar et al. 2022). With approximately 325 different species worldwide, *Grewia* is mainly found in tropical and subtropical regions of Africa, Asia, and Australia (Kumar et al. 2022; Suguna and Umesha 2022). *Grewia* has a long history of traditional use in folk medicinal purposes and food industries due to its source of nutritional values, diverse chemical composition and biological activities (Qamar et al. 2021; Kumar et al. 2022). The bark and other plant parts are used to address various health issues, including diarrhoea, cough, inflammation, and bacterial infections (Ullah et al. 2012; Qamar 2021; Suguna and Umesha 2022). *Grewia* plants are also incorporated into food processing, contributing to the production of preserves, fruit juices, and tea (Sebii et al. 2022) The genus is known to contain various important natural compounds, including flavonoids, alkaloids, triterpenoids, phenols, polysaccharides, and other constituents (Ullah et al. 2012; Suguna and Umesha 2022). These compounds have diverse effects and potential health benefits. Flavonoids, such as catechin, epicatechin, apigenin, luteolin, quercetin, kaempferol, myricetin, and their derivatives, are abundant in *Grewia* and possess antioxidant, anti-inflammatory, anticancer, antidiabetic, and antibacterial properties (Gwatidzo et al. 2018; Kumar et al. 2022). Alkaloids, such as N-methylmicrocosamine, harman, and harman derivatives, have been identified in certain *Grewia* species and exhibit sedative, antispasmodic, antibacterial, and antidiabetic effects (Jaspers et al. 1986; Meena et al. 2017; Kumar et al. 2022). Triterpenoids, such as α-amyrin, lupeol, friedelin, ursolic acid, oleanolic acid, and betulinic acid, are present in *Grewia* and display antibacterial, antidiabetic, antioxidant, anti-inflammatory, antiviral, and anticancer activities (Kumar et al. 2022; Abdelaziz et al. 2023). Phenol and phenolic acids, serving as grewialin, grewin, gallic acid, ellagic acid, caffeic acid, and chlorogenic acid, contribute to the antimalarial, antioxidant, antimicrobial, anti-inflammatory, and anticancer capabilities of *Grewia* (Adebiyi et al. 2017; Koley et al. 2020; Kumar et al. 2022). *Grewia* also contains polysaccharides, such as arabinogalactan, which have immune-enhancing and antibacterial properties (Nep et al. 2011a, 2011b). Additionally, the genus encompasses other constituents like organic acids (citric acid and malic acid) (Meena et al. 2017; Raghu et al. 2023), tannins (Nasrin et al. 2015; Swain et al. 2023), lignans (Ma et al. 2006), and fatty acids (Nyakudya et al. 2015), which are valued for their sour taste and flavor-enhancing characteristics in the food and pharmaceutical industries (Imran et al. 2020; Kumar et al. 2022).

The wide range of bioactive compounds in *Grewia* has attracted the attention of researchers exploring the pharmaceutical and functional food potential of these plants (Imran et al. 2020; Kumar et al. 2022). However, further studies are needed to fully understand the activities and potential applications of these constituents. This research aims to examine the chemical composition of the essential oil extracted from *Grewia bulot* Gagnep. leaves and assess the biological activities associated with the oil, including their antioxidant, anti-inflammatory, and anticancer properties.

2. Results and discussion

Grewia bulot essential oil was obtained from the leaves of the plant, displaying a distinctive red colour with a yield of 0.10% (v/w). Through GC-MS analysis, a comprehensive profile of 78 compounds was successfully identified in the oil, constituting an impressive 94.8% of the overall composition (Table S1). Among the compounds detected, oxygenated sesquiterpenoids emerged as the predominant class, comprising 54.8% of the oil's composition. Oxygenated monoterpenoids accounted for 14.9%, followed by sesquiterpene hydrocarbons (12.5%), non-terpenic compounds (12.3%), and oxygenated diterpenoids (0.3%). Noteworthy compounds found in the oil sample included *α*-cadinol (13.5%), 1,8-cineole (12.7%), 1,10-di-*epi*-cubenol (9.8%), *epi*-*α*-cadinol (6.7%), (*E,E*)-*α*-farnesene (5.9%), (*E*)-citral (4.0%), selin-11-en-4-*α*-ol (4.0%), citronellol isobutanoate (3.9%), and geranic acid (3.7%). Several other compounds were also identified, albeit in lower percentages including diphenyl-disulfide (2.7%), 5-*epi*-7-*epi*-*α*-eudesmol (1.7%), (*E*)-nerolidol (1.7%), *trans*-*β*-guaiene (1.7%), (*Z*)-citral (1.7%), *δ*-cadinol (1.4%), *δ*-selinene (1.4%), hexadecanoic acid (1.4%), *ρ*-*vinyl*-guaiacol (1.3%), *allo*-cedrol (1.2%), geranyl acetate (1.1%), linalool (1.1%), *trans*-sesquisabinene hydrate (1.1%), cadalene (1.0%), benzyl benzoate (1.0%), and elemol (1.0%).

Although several studies have been done on the genus *Grewia*, to the best of our knowledge, this is the first time the chemical composition of *G. bulot* leaf essential oil was reported. Previously, the essential oil extracted from the fresh leaves and stem bark of *G. lasiocarpa* were characterised by phytol (22.6%), *α*-farnesene (8.6%), *n*-hexadecanoic acid (7.2%), farnesol (4.6%) in the leaves, and 2-methylheptadecane (7.2%), heptacosane (7.6%), heptadecane, 2,6,10,14-tetramethyl (7.3%) in the stem bark (Akwu et al. 2019). Interestingly, *α*-farnesene appeared as major component in both *G. bulot* and *G. lasiocarpa* leaf oils.

To ensure that RAW 264.7 cells still survived for further NO production inhibitory evaluation, the cytotoxicity of *Grewia bulot* leaf oil on these cells was tested by MTT assay. At 100 µM, the *G. bulot* leaf oil showed appreciable cytotoxicity since % cell viability value of 78.34 ± 1.80%, whereas the NO production inhibition was insignificant (14.80 ± 0.03%). Thus, *G. bulot* leaf oil did not exhibit NO production inhibitory property in current study.

The *Grewia bulot* leaf oil against the growth of KB, Hep-G2, MCF-7, and SK-LU-1 cell lines was tested by a sulforhodamine B assay, at levels comparable to that of ellipticine as the positive control, and the results are described in Table S3 **AQ2**(Skehan et al. 1990). The *Grewia bulot* leaf oil exhibited cytotoxic effect against KB, Hep-G2, MCF-7, and SK-LU-1 cell lines with IC_{50} values ranging from 44.04 ± 1.47 to 74.20 ± 3.71 µg/mL. Some recent studies related to the cytotoxic activity of the main components in the *G. bulot* essential oil have been announced. *α*-Cadinol (Su and Ho 2013; Sharma et al. 2022), 1,8-cineole (Cai et al. 2021; Sharma et al. 2022), epi-*α*-cadinol, (*E*,*E*)-*α*-farnesene, (*E*)-citral, selin-11-en-4-*α*-ol (Sharma et al. 2022), and citronellyl isobutyrate (Widiyarti et al. 2018) had cytotoxic activities against various human cancer cell lines. Therefore, the oil cytotoxicity against these cancer cell lines in this experiment is due to its constituents. In comparison, the essential oil extracted from the fresh leaves of *G. lasiocarpa* showed cytotoxic activity at 1 mg/mL (IC_{50} = 555.70 µg/mL) against HeLa cells whilst the *G. lasiocarpa* stem bark essential oil exhibited no significant cytotoxic activity (IC_{50} > 1000 µg/mL) (Akwu et al. 2021). The aqueous fruit extract of *G. asiatica* was found to be active on lung (NCI H522), breast (MCF-7), and larynx (HEp-2) cancer cell lines with the IC_{50} values of 59.03, 50.31, and 58.65 µg/mL, respectively. The aqueous leaf extract of this species was active against MCF-7 cancer cell (IC_{50} = 50.37 µg/mL) and HEp-2 cancer cell (IC_{50} = 61.23 µg/mL) (Marya et al. 2011), while the methanol leaf extract was active against four human cancer cell lines including

HL-60, K-562, MCF-7, and Hela, with IC_{50} values of 53.70, 54.90, 199.5 and 177.8 µg/mL, respectively (Periyasamy et al. 2012; Kumar et al. 2022). The cytotoxic activity of the chloroform fraction of *G. bilamellata* (combined leaves, twigs, and stems) against the KB cell line was insignificant, with the ED_{50} > 20 µg/mL (Ma et al. 2006). The methanol extract from the leaves of *G. hirsuta* had a cytotoxic effect on HepG2 cell lines with an IC_{50} value of 15.6 µg/mL (Ema et al. 2013).

Furthermore, the antioxidant activities of *G. bulot* leaf oil were assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay, resulting in an IC_{50} value of 452.65 ± 28.40 µg/mL. The major compounds in the essential oil, such as 1,8-cineole (Cai et al. 2021), farnesene (Çelik et al. 2014), citral (Bouzenna et al. 2017), and selin-11-en-4-*α*-ol (Al-Qudah et al. 2014) had shown antioxidant properties. The presence of these constituents mainly contributed to the antioxidant activities of *G. bulot* leaf oil. In similar manner, the methanol extract of *G. villosa* demonstrated weak DPPH scavenging effects at various concentrations, comparable to the standard vitamin E (Hegazy et al. 2019). The methanol and acetone extracts of *G. optiva* leaves even did not exhibit antioxidant activity (Arora 2011). The antioxidant potential of *G. tenax* was evaluated by assessing the syrup, jam, and seed extracts. Among these, the seed extract displayed the highest antioxidant activity (Suliman et al. 2018). *G. tenax* exhibited the highest antioxidant potential (85.49 ± 2.68%), while *G. tilifolia* (76.11 ± 1.77%) and *G. asiatica* (82.5 ± 5.66%) displayed comparatively lower antioxidant activities (Sharma et al. 2016). The methanol extract of *G. sapida* was also tested for its antioxidant activity using the DPPH assay, demonstrating an IC_{50} value of 257.666 ± 2.516 µg/mL (Islary et al. 2016). Furthermore, the crude chloroform and methanol extracts from the stem bark of *G. lasiocarpa* exhibited significant antioxidant activity, with the highest inhibition observed at 75.19% and 92.94% for DPPH and FRAP assays, respectively (Akwu et al. 2021). Regarding *G. asiatica*, the methanol extract of its fruit displayed considerable antioxidant activity (Srivastava et al. 2012). The petroleum ether fraction of *G. abutilifolia* leaf exhibited the highest DPPH scavenging activity (IC_{50} = 3.82 ± 0.055 µg/mL) (Salam and Rafe 2018). Comparisons between the DPPH radical scavenging activities of *G. bulot* and other reported *Grewia* species suggested that *G. bulot* generally exhibited stronger antioxidant activity. These findings highlight the potential of *G. bulot* as a valuable source of ethnic medicinal plants for the development of novel antioxidant therapies.

3. Conclusions

This study presents a comprehensive analysis of the chemical composition and *in vitro* biological activities of the essential oil extracted from *Grewia bulot* leaves. The essential oil exhibited a diverse array of chemical components, with prominent classes including oxygenated sesquiterpenoids (54.8%), oxygenated monoterpenoids (14.9%), sesquiterpene hydrocarbons (12.5%), non-terpenic compounds (12.3%), and oxygenated diterpenoids (0.3%). Among the identified compounds, *α*-cadinol (13.5%), 1,8-cineole (12.7%), 1,10-di-epi-cubenol (9.8%), epi-*α*-cadinol (6.7%), and (*E*,*E*)-*α*-farnesene (5.9%) were found in significant proportions. The essential oil demonstrated noteworthy antioxidant activity as assessed by the DPPH radical scavenging assay, indicating its potential as an effective scavenger of free radicals. Furthermore, the essential oil exhibited significant anticancer effects against four human cancer cell lines (KB, Hep-G2, MCF-7, and SK-LU-1), suggesting its potential as an agent for cancer treatment. These findings underscore the bioactive nature of *Grewia bulot* essential oil and highlight its potential for therapeutic applications, particularly in the field of antioxidant therapy and cancer treatment. However, further investigations are warranted to elucidate the underlying mechanisms responsible for these observed effects and to identify specific bioactive compounds within the essential oil that contribute to its remarkable properties.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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