Exosome-like nanoparticles from the Okinawan medicinal plant *Talinum fruticosum* exhibit subset-specific bioactivities *in vitro*: Suppression of IL-6 production and reduction of intracellular reactive oxygen species

YASUNARI KAGEYAMA[1-3], KAZUNORI NAMBA[2], YUKI AKAMATSU[2], TOMOKA EBISUI[2], KOICHI AIDA[1] and TSUTOMU NAKAMURA[1,2]

[1]Takanawa Clinic, Tokyo 108-0074, Japan; [2]IDO Medical Co., Ltd., Tokyo 140-0002, Japan; [3]Tokai University Hospital, Isehara, Kanagawa 259-1193, Japan

Received September 23, 2025; Accepted February 19, 2026

DOI: 10.3892/br.2026.2145

Abstract. Plant-derived exosome-like nanoparticles (ELNs) exhibit diverse physiological activities across biological kingdoms. *Talinum fruticosum* (L.) Juss., known in Okinawa as 'Shibiran', is a traditional edible medicinal plant that is considered a food-based remedy. Okinawa, one of the five globally recognized 'blue zones', is renowned for its longevity and distinctive health practices. In Okinawan culture, the boundary between food and medicine is often blurred, and medicinal plants such as *T. fruticosum* are regularly incorporated into the daily diet for their health-promoting properties. However, the biological basis of the health benefits remains poorly understood. To assess its potential pharmacological value, ELNs from *T. fruticosum* (TfELNs) were isolated and characterized, and their key biological activities were examined. TfELNs were isolated from fresh leaves via ultracentrifugation followed by sucrose density gradient centrifugation. Their size and morphology were analyzed using nanoparticle tracking analysis and transmission electron microscopy. Subsequently, anti-inflammatory effects were evaluated in human THP-1 macrophages stimulated with lipopolysaccharide (LPS) using a multiplex cytokine immunoassay. Antioxidant activity was assessed in THP-1 macrophages under hydrogen peroxide-induced oxidative stress, with intracellular reactive oxygen species (ROS) levels measured using a DCFH-DA fluorescence assay. TfELNs were identified as spherical vesicles with typical exosome-like features, and they exhibited no notable cytotoxicity, thus indicating high biocompatibility. Notably, distinct subset-specific bioactivities were observed: The B2 fraction significantly suppressed LPS-induced interleukin-6 (IL-6) production in THP-1 macrophages, whereas the B1 fraction markedly reduced ROS levels under oxidative stress. These results suggested that TfELNs may exert selective anti-inflammatory and antioxidant activities, highlighting their potential utility in managing IL-6-associated inflammatory and oxidative stress-related conditions. These subset-specific activities suggest a unique pharmacological profile of TfELNs that could inspire future therapeutic applications. Furthermore, these findings may partially explain the traditional medicinal value attributed to *T. fruticosum* and underscore its relevance in the ethnopharmacological context of Okinawan healthy longevity.

Correspondence to: Dr Tsutomu Nakamura, Takanawa Clinic, 4-23-6 Takanawa, Minato, Tokyo 108-0074, Japan
E-mail: nakamurachibiko@gmail.com

Abbreviations: ELNs, exosome-like nanoparticles; IL, interleukin; LPS, lipopolysaccharide; NTA, nanoparticle tracking analysis; PBS, phosphate-buffered saline; ROS, reactive oxygen species; TEM, transmission electron microscopy; TfELNs, ELNs from *Talinum fruticosum*

Key words: Talinum fruticosum, extracellular vesicles, exosomes, ELNs, IL-6, inflammation, ROS, oxidative stress

Introduction

Growing evidence indicates that many animal cell types secrete nano-sized membranous vesicles, known as exosomes, that mediate intercellular communication (1,2). Exosomes deliver bioactive cargo molecules, including proteins, nucleic acids, and small-molecule metabolites, via paracrine and endocrine mechanisms, thereby modulating the metabolism and biological functions of recipient cells (1,2). In recent years, it has become evident that plant cells also produce extracellular vesicles known as exosome-like nanoparticles (ELNs), which share morphological and functional characteristics with animal exosomes (3). These plant ELNs are enriched with natural compounds such as flavonoids, terpenoids, and alkaloids, and exhibit cross-kingdom biological activities in mammalian cells, including anti-inflammatory, antioxidant, anti-tumor, anti-senescent, and neuroprotective effects (4,5).

Talinum fruticosum (L.) Juss., an edible medicinal plant belonging to the family *Talinaceae*, is distributed across tropical and subtropical regions, particularly in West Africa,

South America, and South/Southeast Asia (6-9). Notably, major botanical authorities such as Plants of the World Online and the Flora of North America treat *T. fruticosum* as a synonym or conspecific of *T. triangulare* (10,11). Studies on *T. triangulare* have reported its anti-inflammatory and immunomodulatory effects in animal models, which we cite here as supportive background for the pharmacological relevance of *T. fruticosum* (12,13). Similarly, *T. paniculatum* is treated as closely related and/or occasionally listed under the same taxonomic treatment as *T. fruticosum* in certain plant classification databases, including the Global Biodiversity Information Facility (6,14). In ethnobotanical contexts, *T. paniculatum* is often confused with *T. fruticosum* due to their morphological similarity, and both species are traditionally used for comparable medicinal purposes. Although scientific studies on *T. paniculatum* are limited, emerging evidence indicates its cardioprotective potential in rat models through antioxidant activity and angiotensin-converting enzyme inhibition (15).

Okinawa, Japan, a region globally recognized as a 'blue zone' for its high longevity, has a unique culture in which the boundary between food and medicine has traditionally been blurred in daily life (16-19). *T. fruticosum* (locally known as 'Shibiran') is both cultivated and naturalized in Okinawa (6), and has long been recognized as a traditional food-medicine plant. It has been commonly consumed in the Okinawan diet as a leafy vegetable for maintaining general health and traditionally used as a folk remedy for managing ailments such as gastric discomfort, diarrhea, cough, asthma, and inflammatory swelling (20). These uses are consistent with Japanese-language ethnobotanical records that document its role in the food-medicine continuum deeply rooted in Okinawan culture. The Okinawan use of *T. fruticosum* highlights a form of empirical knowledge that has long informed local health practices, yet remains underrepresented in English-language scientific literature.

While *T. fruticosum* has long been valued as an edible medicinal plant, the biological basis of its health benefits remains largely unexplored. In this study, as part of our broader ethnopharmacological project focusing on Okinawan medicinal plants, we identified ELNs from *T. fruticosum* leaves (TfELNs). We hypothesized that TfELNs may contribute to the health-promoting effects of *T. fruticosum* and aimed to evaluate their potential immunomodulatory and antioxidant activities using a macrophage-based *in vitro* assay.

Materials and methods

Plant material identification and source information. Fresh aerial parts of *Talinum fruticosum* (L.) Juss. were obtained from Atarasu market, a local farmers' market located in Hirara-Nishizato, Miyakojima-shi, Okinawa, Japan, on July 1, 2024. This market is known for direct sales of freshly harvested local produce by regional farmers. The plants were harvested on the same day at the Uchima farm, located in Hirara-Shimozato, Miyakojima-shi, Okinawa, Japan.

Plant identification was initially carried out by the authors based on morphological characteristics described in the published literature and authoritative botanical databases (e.g., Plants of the World Online). Key features included leaf shape, flower bud morphology, and the characteristic triple-ridged structure of the rachis. Detailed photographic documentation of the plant is provided in Figs. S1 and S2.

To further authenticate the taxonomic identity of the plant material used for TfELN preparation, DNA barcoding was conducted using two plastid loci, *rbcL* and *matK*. DNA extraction, PCR amplification, and Sanger sequencing were outsourced to Fasmac Co., Ltd.. For *rbcL*, PCR was performed using primers rbcLa-F/rbcLa-R, and a 553-bp sequence excluding primer regions was obtained. For *matK*, PCR was performed using primers 390F/1326R, and an 852-bp sequence excluding primer regions and adjacent low-quality ends was obtained from a 942-bp amplicon. Primer sequences were obtained from the Barcode of Life Data (BOLD) Systems Primer Database.rbcLa-F: 5'-ATGTCACCACAAACAGAG ACTAAAGC-3'; rbcLa-R: 5'-GTAAAATCAAGTCCACCR CG-3' (R=A/G); 390F: 5'-CGATCTATTCATTCAATATTT C-3'; 1326R: 5'-TCTAGCACACGAAAGTCGAAGT-3'.

Sequence similarity searches were performed using BLASTN against the NCBI nucleotide database. The *rbcL* and *matK* sequences obtained in this study showed high similarity to publicly available *T. fruticosum* reference sequences (Figs. S3 and S4). Because plastid barcode loci may show limited interspecific divergence in closely related taxa, the final species identification was made by integrating the DNA barcoding results with the diagnostic morphological characteristics shown in Figs. S1 and S2 (including the three longitudinal ridges on the rachis). Accordingly, the plant material used in this study was treated as *T. fruticosum* in subsequent experiments.

Preparation of TfELNs. Fresh *T. fruticosum* plant (Fig. 1A) was obtained from a local farm in Miyako Island, Okinawa Prefecture, Japan. TfELNs were prepared as previously described with slight modifications (21). Leaves were washed, briefly sterilized with 70% ethanol, and homogenized using a kitchen blender (Acasas A-1200; Eco Setsubi Inc.). The homogenate was centrifuged at 16,000 x g for 40 min at 4˚C (Avanti J-26S XP; Beckman Coulter). The supernatant was then centrifuged at 170,000 x g for 90 min at 4˚C (Optima XE-90 Ultracentrifuge; Beckman Coulter), and the pellet was resuspended in phosphate-buffered saline (PBS). Subsequently, 60/45/30/8% sucrose density gradient ultracentrifugation was performed at 155,000 x g for 90 min at 4˚C. Two visible bands at the 8/30% (B1) and 30/45% (B2) interfaces were collected using a 20G syringe needle (TERUMO). The B1 and B2 fractions were diluted with distilled water and centrifuged at 170,000 x g for 90 min at 4˚C. The pellet was resuspended in 1 ml of 30% sucrose solution for cryoprotection. The purified sample was filtered through a 0.22-μm polyethersulfone membrane filter (Merck Millipore) and stored at -80˚C.

Characterization of TfELNs. Size distribution and particle concentration of TfELNs were measured using nanoparticle tracking analysis (NTA) (NanoSight NS300; Malvern Panalytical). Protein concentration was determined with the Qubit Protein Assay Kit (Thermo Fisher Scientific, Inc.). The yield was calculated as the number of particles and proteins in TfELNs per weight of fresh *T. fruticosum* leaf. TfELN

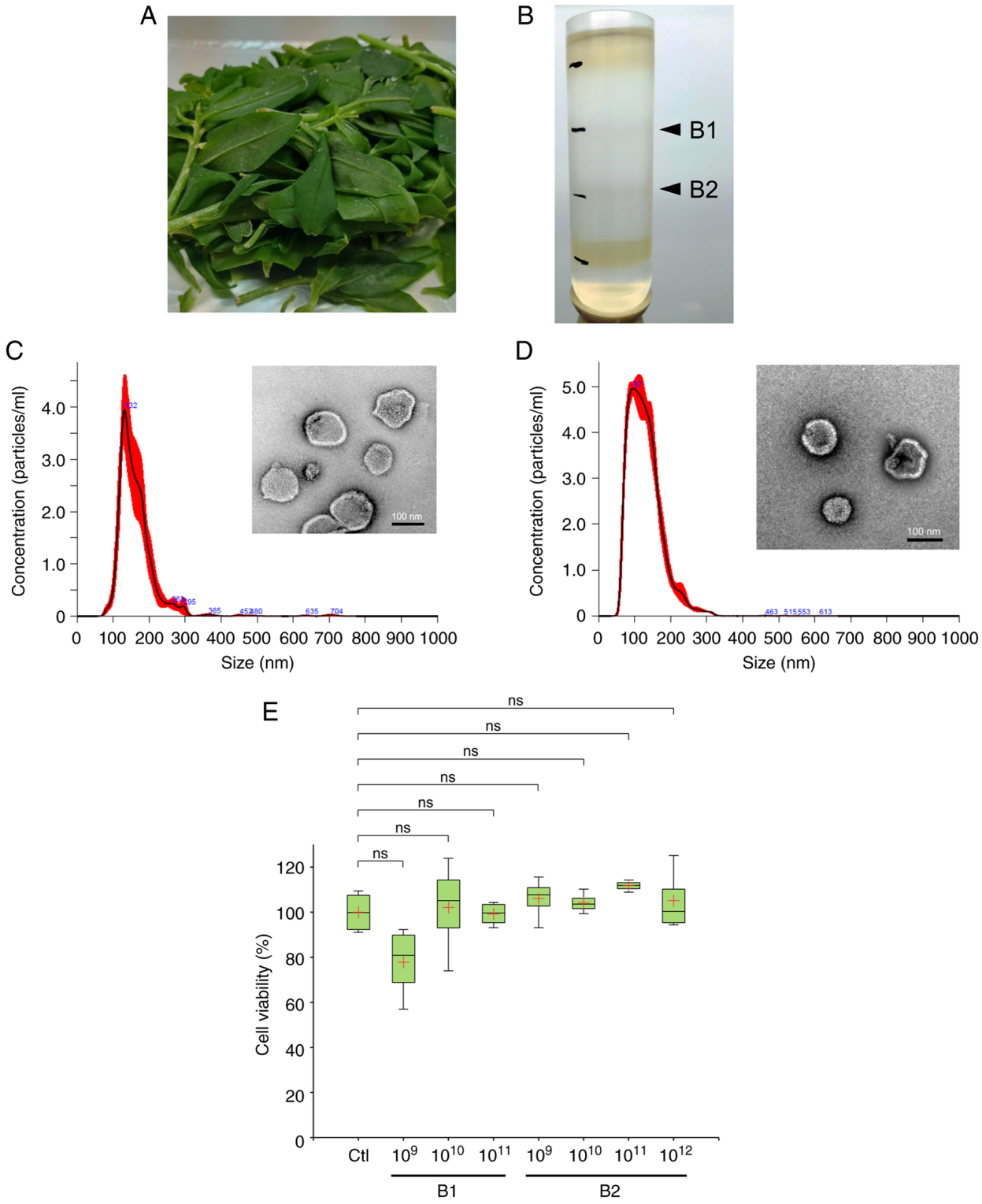

Figure 1. Isolation and characterization of TfELNs. (A) Fresh *Talinum fruticosum* leaves. (B) Purification of TfELNs using sucrose density gradient ultracentrifugation: Upper and lower arrowheads indicate TfELN B1 and B2 bands, respectively. Particle size distributions of purified TfELN (C) B1 and (D) B2, determined by NTA. Representative TEM images at 50,000x magnification are shown as insets in each panel. Scale bar, 100 nm. (E) Cytotoxicity of TfELN B1 and B2 in THP-1 macrophages. Box plots: horizontal line, median; red cross, mean. Brackets with 'ns' (not significant) indicate specific comparisons between the control group and each treatment group (P>0.05). Additionally, no statistically significant differences were found between any other pairs in the post hoc analysis (all P>0.05). TfELN, *Talinum fruticosum*-derived exosome-like nanoparticle; NTA, nanoparticle tracking analysis; TEM, transmission electron microscopy.

morphology was analyzed using transmission electron microscopy (TEM) (JEM-2000EX; JEOL) by the Hanaichi UltraStructure Research Institute (Okazaki).

Preparation of THP-1 macrophages. The THP-1 human monocytic cell line was purchased from KAC Co., Ltd. (EC88081201-FF) and cultured in RPMI-1640 medium

(FUJIFILM Wako Pure Chemical) supplemented with 10% fetal bovine serum (MP Biomedicals), 1% GlutaMAX (Thermo Fisher Scientific, Inc.), and 1X Antibiotic-Antimycotic (Thermo Fisher Scientific, Inc.). To induce macrophage differentiation, THP-1 cells were suspended in RPMI-1640 medium containing 160 nM phorbol 12-myristate 13-acetate (Abcam) and seeded at $2x10^4$ cells/well in a 96-well plate (SANPLATEC) (22,23). After 48 h incubation at 37˚C, adherent THP-1 macrophages were washed with PBS and incubated for an additional 24 h before lipopolysaccharide (LPS) stimulation.

Cytotoxicity of TfELNs. THP-1 macrophages were treated with TfELNs (10^9-10^{12} particles/ml) in RPMI-1640 medium for 48 h. Assays with 10^{12} particles/ml of TfELN B1 could not be performed due to its extremely low yield. Cell viability was assessed using the Cell Counting Kit-8 (Dojindo Laboratories).

In vitro cytokine release assay. THP-1 macrophages were pretreated with TfELNs (10^{11} particles/ml) in RPMI-1640 medium for 24 h and then stimulated with 0.5 mg/ml *Salmonella typhimurium*-derived LPS (Fujifilm Wako Pure Chemical) for 24 h. For dose-dependency experiments, TfELNs (10^9-10^{12} particles/ml) were used. Dexamethasone, a steroidal anti-inflammatory drug, was used as a positive control. Cytokine levels in the culture supernatant were measured using the V-PLEX Pro-inflammatory Panel 1 Human Kit (Meso Scale Discovery) and the MESO QuickPlex SQ 120MM (Meso Scale Discovery).

Intracellular reactive oxygen species (ROS) assay. THP-1 macrophages were pretreated with TfELNs (10^8-10^{11} particles/ml) in RPMI-1640 medium for 24 h and then stimulated with 1 mM hydrogen peroxide (H_2O_2) for 4 h. The intracellular ROS levels were measured using a ROS Assay Kit (Photo-oxidation Resistant DCFH-DA; Dojindo Laboratories) according to the manufacturer's instructions, with a Spark multimode microplate reader (Tecan Japan Co., Ltd.).

Statistical analyses. All statistical analyses were performed using XLSTAT (Lumivero). Outlier detection was not performed; all collected data were included. Normality was tested using the Shapiro-Wilk test. Homogeneity of variances was assessed using either Bartlett's or Brown-Forsythe test, depending on the distribution of the data.

Based on these results, appropriate parametric or non-parametric methods were applied as follows. Data presented in Fig. 1E were analyzed using Welch's ANOVA, followed by the Games-Howell post hoc test. Fig. 2A data were analyzed with the Kruskal-Wallis test, followed by the Conover-Iman test with Bonferroni correction. For Fig. 2B (no LPS stimulation), the Kruskal-Wallis test was used, followed by Dunn-Bonferroni post hoc test. For Fig. 2B (LPS stimulation), Welch's ANOVA was performed. While the Games-Howell test served as the primary analysis for robust, exploratory all-pairs comparisons, Dunnett's test was specifically employed for comparisons against the control group to provide optimized statistical power for these primary reference points. Group comparisons for Fig. 3A were conducted using the Kruskal-Wallis test, with Dunn-Bonferroni post hoc test. For Fig. 3B, Welch's ANOVA was applied, followed by the Games-Howell post hoc test. Statistical significance was set at $P<0.05$.

Results

Isolation and characterization of TfELNs. We successfully isolated two distinct fractions of TfELNs, referred to as TfELN B1 and B2, from *T. fruticosum* leaves (Fig. 1B). The average yields per gram of fresh *T. fruticosum* leaves were $2.94x10^9$ particles (1.36 μg of protein) for TfELN B1 and $5.26x10^{10}$ particles (12.4 μg of protein) for TfELN B2. NTA revealed that the size distribution of TfELN B1 ranged from 117.8 to 212.6 nm (D10-D90), with mean and mode particle sizes of 162.9 nm and 131.5 nm, respectively (Fig. 1C). TfELN B2 had a slightly smaller particle size distribution, ranging from 78.5 to 185.1 nm (D10-D90), with a mean of 128.8 nm and a mode of 96.1 nm (Fig. 1D). TEM analysis confirmed that both TfELN B1 and B2 exhibited spherical vesicle morphology typical of exosomes, with diameters ranging from approximately 50 to 110 nm (insets in Fig. 1C and D). No notable morphological differences were observed between the two fractions.

TfELNs exhibit subset-specific anti-inflammatory effects. We first evaluated the cytotoxicity of TfELNs on THP-1-derived macrophages. Treatment with various concentrations of TfELN B1 (10^9-10^{11} particles/ml) and B2 (10^9-10^{12} particles/ml) did not significantly affect cell viability (Fig. 1E), indicating the high biocompatibility of TfELNs. Due to the low yield of B1, 10^{11} particles/ml was used as the maximum feasible dose for subsequent assays.

Next, the anti-inflammatory potential of TfELNs was assessed using LPS-stimulated THP-1 macrophages. LPS stimulation markedly increased the production of inflammatory cytokines compared with the unstimulated control (Fig. 2A). Among the tested cytokines, interleukin-6 (IL-6) production was significantly reduced by pretreatment with TfELN B2 (53.5% inhibition), whereas TfELN B1 induced a modest but non-significant reduction (34.6% inhibition). Neither fraction significantly affected the production of other pro-inflammatory cytokines. Dose-dependent suppression of IL-6 production was observed upon treatment with increasing concentrations of TfELN B2 (10^9-10^{12} particles/ml) (Fig. 2B). IL-6 inhibition was statistically significant only at 10^{11} and 10^{12} particles/ml. These findings suggest that TfELNs, particularly the B2 subset, exert anti-inflammatory effects through selective suppression of IL-6 production in activated macrophages.

TfELNs exhibit subset-specific antioxidant effects. We next examined the antioxidant effects of TfELNs. THP-1 macrophages were pretreated with TfELNs (10^8-10^{11} particles/ml) and then exposed to H_2O_2 to induce oxidative stress, with intracellular ROS levels quantified using DCFH-DA fluorescence. Pretreatment with B1 resulted in a significant reduction in ROS levels compared with the control at particle concentrations of 10^9-10^{11} (Fig. 3A). In contrast, B2 did not show any significant effect on ROS levels across the tested particle concentrations (Fig. 3B), demonstrating the subset-specific antioxidant effects of TfELNs.

Discussion

In this study, we identified ELNs derived from *T. fruticosum* leaves and demonstrated their selective inhibitory effect on IL-6 production in an LPS-induced *in vitro* inflammation

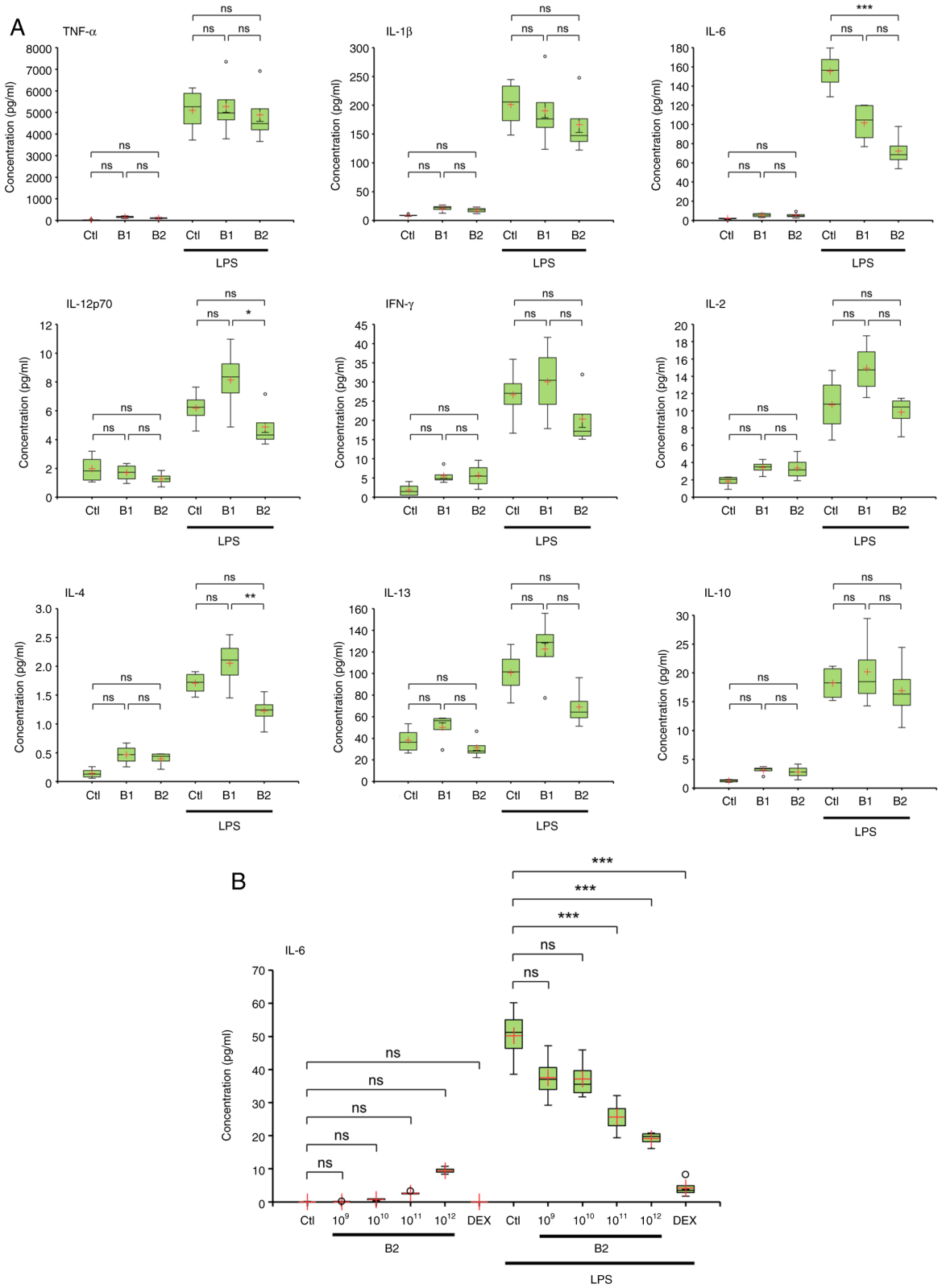

Figure 2. Selective inhibition of IL-6 production by TfELNs. (A) Levels of representative pro-inflammatory cytokines in LPS-stimulated THP-1 macrophages, with or without pretreatment (10^{11} particles/ml) of TfELN B1 or B2. Only IL-6 was significantly suppressed. (B) Dose-response of IL-6 inhibition by TfELN B2. Box plots: horizontal line, median; red cross, mean. To avoid visual clutter, brackets are only shown for significant differences or comparisons with the control group; all other pairwise comparisons were non-significant (P>0.05). Statistical significance is indicated as follows: *P<0.05; **P<0.01; ***P<0.001; ns, not significant. TfELN, *Talinum fruticosum*-derived exosome-like nanoparticle; IL, interleukin; LPS, lipopolysaccharide.

model. TfELNs suppressed IL-6 secretion in a dose-dependent manner without affecting other cytokines or inducing cytotoxicity, indicating a specific immunomodulatory effect rather than a general anti-inflammatory or cytotoxic response. IL-6 is a key mediator of inflammation and contributes to the pathogenesis of several autoimmune diseases (24), including

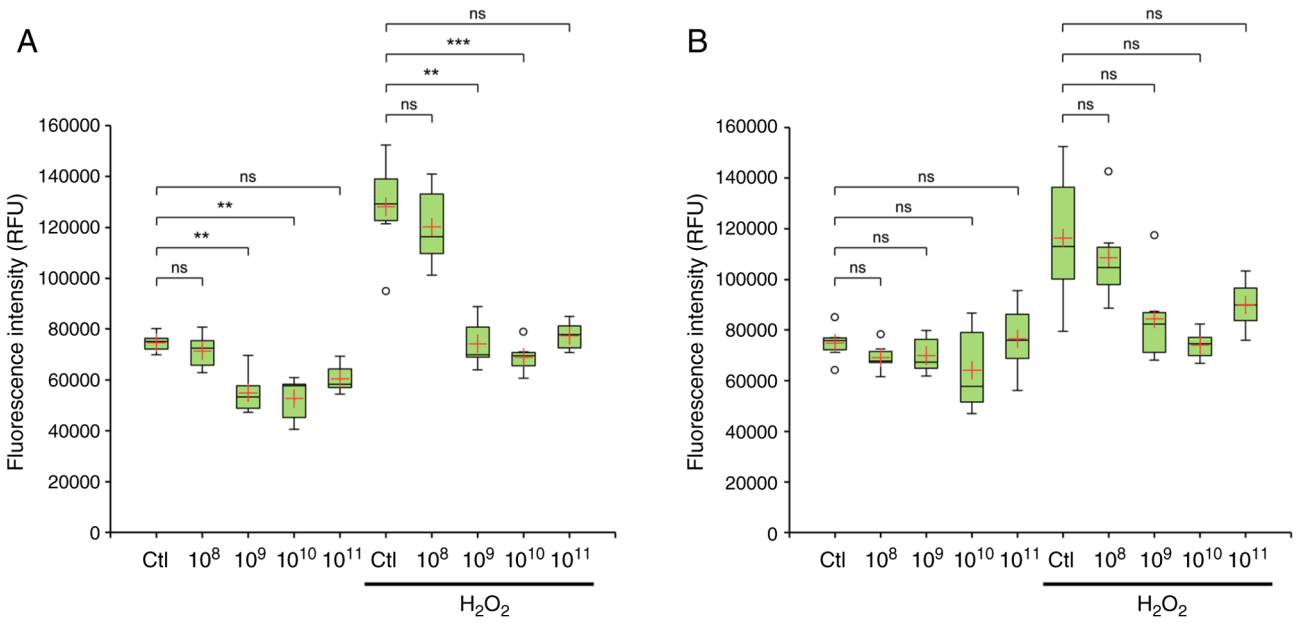

Figure 3. Differential antioxidant activity of TfELN subsets. Intracellular ROS levels in H_2O_2-treated THP-1 macrophages were measured using DCFH-DA fluorescence intensity, and values are expressed as RFU. (A) Pretreatment with B1 significantly reduced ROS levels, (B) whereas pretreatment with B2 had no detectable antioxidant effect. Box plots: horizontal line, median; red cross, mean. To avoid visual clutter, brackets are only shown for significant differences or comparisons with the control group; all other pairwise comparisons were non-significant (P>0.05). Statistical significance is indicated as follows: *P<0.05; **P<0.01; ***P<0.001; ns, not significant. TfELN, *Talinum fruticosum*-derived exosome-like nanoparticle; ROS, reactive oxygen species; RFU, relative fluorescence units; DCFH-DA, 2',7'-dichlorodihydrofluorescein diacetate.

rheumatoid arthritis (25), systemic lupus erythematosus (26), and Castleman disease (27). Its excessive production is also associated with severe systemic inflammation, such as cytokine storms in COVID-19 and sepsis (28,29), and supports the growth and survival of multiple myeloma and ovarian endometriosis cells (30,31). Although pharmacological inhibitors for IL-6 signaling are clinically available (32), their use is limited by high cost and the potential for adverse effects with long-term treatment (33). In contrast, TfELNs are derived from edible plant material using simple preparation methods, offering a potentially safer and more accessible approach for modulating IL-6-driven inflammation.

Another intriguing finding of this study is the functional divergence between the two TfELN subsets: B1 exhibited significant antioxidant activity without detectable anti-inflammatory activity, whereas B2 displayed anti-inflammatory activity without detectable antioxidant effects. This differential activity indicates that TfELN functions are subset-specific and may reflect differences in their molecular cargo, such as distinct profiles of proteins, lipids, or small RNAs (34,35). Such cargo differences could influence recipient macrophages following cellular uptake, for example by modulating inflammatory signaling and oxidative stress-response pathways. In other edible plant-derived ELNs/EVs, reported cargos commonly include small RNAs (e.g., miRNAs), membrane lipids enriched in phospholipids such as phosphatidic acid and phosphatidylcholine, and a limited set of proteins related to stress responses and metabolism (4,5,34,35). In addition, some plant ELNs have been reported to carry plant-derived bioactive small molecules (e.g., flavonoids or isothiocyanates) depending on the source species, which could in principle contribute to anti-inflammatory and/or antioxidant phenotypes (4,5,34,35). Although cargo composition was not assessed here, fraction-dependent differences may underlie the divergent effects of B1 and B2, with potential implications for tailoring TfELN subsets to specific biological contexts. The selective antioxidant effect of B1 could be particularly relevant for mitigating oxidative stress-related conditions, while the anti-inflammatory properties of B2 may play a key role in modulating IL-6-driven immune responses. This functional specialization suggests that isolating or enriching specific EV subsets could enhance therapeutic efficacy for targeted applications (4,36). Further studies are warranted to identify the molecular determinants underlying these subset-specific activities and to explore their potential in *in vivo* applications.

Plant-derived ELNs with anti-inflammatory activity have been reported from multiple edible and medicinal plants. For example, ELNs isolated from *Solanum nigrum* berries were shown to attenuate IL-6-related inflammatory responses in LPS-stimulated RAW264.7 macrophages (37), supporting the feasibility of IL-6 modulation by plant EV preparations. In our study, TfELN B2 selectively reduced LPS-induced IL-6 secretion in THP-1 macrophages, whereas other measured pro-inflammatory cytokines were not significantly altered under the same conditions. This profile is consistent with a targeted immunomodulatory effect rather than broad cytokine suppression. Notably, dose reporting varies across the literature (e.g., protein-based μg/ml vs. particle-based particles/ml), and such differences, together with variation in plant source material and isolation workflows, may contribute to apparent differences in effective dose ranges between studies.

The discovery of TfELNs sheds light on why *T. fruticosum* has long been valued in Okinawa as both food and medicine. The presence of ELNs with specific biological activity provides a molecular rationale for its traditional use and supports the concept that there are 'no clear boundaries between food and medicine' (16-19). Moreover, this finding may have broader

relevance, as *T. fruticosum* and similar edible medicinal plants are traditionally used in diverse cultures worldwide (7-9). By elucidating the functional role of ELNs, our results offer a new perspective for interpreting traditional plant-based therapies within a modern biomedical framework.

This study has several limitations. The specific molecular cargos responsible for IL-6 suppression and ROS reduction remain unidentified, and comprehensive multi-omics profiling will be required to elucidate their mechanisms. We also note that dedicated stability testing of TfELNs under storage or physiological conditions was not performed in this study and will be important for future translational applications. Moreover, although marker-based characterization is commonly applied to mammalian exosomes, universally accepted marker panels for plant-derived ELNs have not yet been established, which currently limits standardized comparisons across studies (38,39). In addition, other potentially relevant biological activities, such as anti-senescent or neuroprotective effects, were not investigated in the present study. The current work was designed as an initial, subset-focused *in vitro* evaluation of selective IL-6 suppression and ROS reduction; broader profiling across additional endpoints and/or cell types will be an important direction for future studies. *In vivo* studies will also be critical to establish the therapeutic relevance and safety of TfELNs under physiological conditions. Future research should further explore how TfELNs contribute to the health-promoting properties of *T. fruticosum*, particularly within the framework of Okinawa's longevity culture and its broader ethnopharmacological significance.

In conclusion, this study demonstrates that exosome-like nanoparticles from *T. fruticosum* leaves exhibit subset-specific activities: B2 selectively inhibits IL-6 production, while B1 exerts significant antioxidant effects *in vitro*. These findings provide a scientific basis for its ethnopharmacological use in Okinawan practices and highlight the broader potential of similar edible medicinal plants worldwide.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YK conceptualized the study, acquired funding, provided key study materials and resources, supervised the work, and reviewed and edited the manuscript. KN curated and managed the data, performed the formal/statistical analyses, conducted the investigations, developed the methodology, administered the project, validated the results, prepared the figures and tables, drafted the original manuscript, and reviewed and edited the manuscript. YA performed the formal/statistical analyses, conducted the investigations, developed the methodology, validated the results, and reviewed and edited the manuscript. TE conceptualized the study, curated and managed the data, administered the project, and reviewed and edited the manuscript. KA conceptualized the study, acquired funding, supervised the work, and reviewed and edited the manuscript. TN conceptualized the study, performed the formal/statistical analyses, supervised the work, validated the results, prepared the figures and tables, drafted the original manuscript, and reviewed and edited the manuscript. KN and TN confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

Yasunari Kageyama is the chief executive officer of IDO Medical Co., Ltd. (Tokyo, Japan) and owns shares of the company. Kazunori Namba, Yuki Akamatsu, and Tomoka Ebisui are employees of IDO Medical Co., Ltd. Koichi Aida is the representative director of Takanawa Clinic (Tokyo, Japan). Tsutomu Nakamura is the director of IDO Medical Co., Ltd. and the scientific adviser of Takanawa Clinic.

References

1. Kalluri R and LeBleu VS: The biology, function, and biomedical applications of exosomes. Science 367: eaau6977, 2020.
2. van Niel G, D'Angelo G and Raposo G: Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol 19: 213-228, 2018.
3. Zeng YB, Deng X, Shen LS, Yang Y, Zhou X, Ye L, Chen S, Yang DJ and Chen GQ: Advances in plant-derived extracellular vesicles: Isolation, composition, and biological functions. Food Funct 15: 11319-11341, 2024.
4. Lian MQ, Chng WH, Liang J, Yeo HQ, Lee CK, Belaid M, Tollemeto M, Wacker MG, Czarny B and Pastorin G: Plant-derived extracellular vesicles: Recent advancements and current challenges on their use for biomedical applications. J Extracell Vesicles 11: e12283, 2022.
5. Zhu Y, Zhao J, Ding H, Qiu M, Xue L, Ge D, Wen G, Ren H, Li P and Wang J: Applications of plant-derived extracellular vesicles in medicine. MedComm (2020) 5: e741, 2024.
6. Global Biodiversity Information Facility (GBIF): *Talinum fruticosum* (L.) Juss. Available: https://www.gbif.org/species/3084670. Accessed September 19, 2025.
7. Manikandan K, Balaji T and Dhanushkodi V: *Talinum fruticosum*: A potential multi-value plant: A review. Agric Rev 46: 416-422, 2025.
8. Dinesh A and Kumar A: A review on bioactive compounds, ethnomedicinal importance and pharmacological activities of *Talinum triangulare* (Jacq.) Willd. Chem Biodivers 20: e202301079, 2023.
9. Barman D, Puro KN, Boruah JLH, Kumar D, Medhi K, Mazumder B and Baishya R: *Talinum triangulare* (Jacq.) Willd: A review of its traditional uses, phytochemistry, and pharmacology along with network pharmacology analysis of its components and targets. Food Chem Adv 5: 100768, 2024.
10. Plants of the World Online (POWO): *Talinum fruticosum* (L.) Juss. Available: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:1182764-2. Accessed September 19, 2025.

11. Flora of North America (FNA): *Talinum fruticosum*. Available: http://floranorthamerica.org/Talinum_fruticosum. Accessed September 19, 2025.
12. Inwang UA, Ben EE, Uchewa OO, Nwuzor EO, Nwaji AR and Umoh EA: Anti-oxidant and anti-inflammatory properties of *Talinum triangulare* methanol leaf extract on cadmium-induced cognitive dysfunction in male wistar rat. Nat Prod Commun 19: 1-10, 2024.
13. Oladele JO, Ojuederie OB, Oladele OT, Ajayi EIO, Olaniyan MD, Atolagbe OS, Okoro OE, Adewale OO and Oyeleke OM: Gastroprotective mechanism of *Talinum triangulare* on ethanol-induced gastric ulcer in Wistar rats via inflammatory, antioxidant, and H^+/K^+-ATPase inhibition-mediated pathways. Drug Chem Toxicol 48: 281-293, 2025.
14. Global Biodiversity Information Facility (GBIF): *Talinum paniculatum* Moench. Available: https://www.gbif.org/species/7906855. Accessed September 19, 2025.
15. Souto CGRG, Lorençone BR, Marques AAM, Palozi RAC, Romão PVM, Guarnier LP, Tirloni CAS, Dos Santos AC, Souza RIC, Zago PMJJ, *et al*: Cardioprotective effects of *Talinum paniculatum* (Jacq.) Gaertn. in doxorubicin-induced cardiotoxicity in hypertensive rats. J Ethnopharmacol 281: 114568, 2021.
16. Miyagi S, Iwama N, Kawabata T and Hasegawa K: Longevity and diet in Okinawa, Japan: The past, present and future. Asia Pac J Public Health 15 (Suppl 1): S3-S9, 2003.
17. Willcox BJ, Willcox DC, Todoriki H, Fujiyoshi A, Yano K, He Q, Curb JD and Suzuki M: Caloric restriction, the traditional Okinawan diet, and healthy aging: The diet of the world's longest-lived people and its potential impact on morbidity and life span. Ann NY Acad Sci 1114: 434-455, 2007.
18. Willcox DC, Willcox BJ, Todoriki H and Suzuki M: The Okinawan diet: Health implications of a low-calorie, nutrient-dense, antioxidant-rich dietary pattern low in glycemic load. J Am Coll Nutr 28 (Suppl 1): 500S-516S, 2009.
19. Willcox DC, Scapagnini G and Willcox BJ: Healthy aging diets other than the Mediterranean: A focus on the Okinawan diet. Mech Ageing Dev 136-137: 148-162, 2014.
20. Subtropical Plant Resources Database, University of the Ryukyus: *Talinum paniculatum* Gaertn. Available: https://iicc.skr.u-ryukyu.ac.jp/plant/br/720.php. Accessed September 19, 2025.
21. Kim J, Li S, Zhang S and Wang J: Plant-derived exosome-like nanoparticles and their therapeutic activities. Asian J Pharm Sci 17: 53-69, 2022.
22. López-López S, Castro-Robles B, García-Flores N, Díaz-Guerra MJM, Arias-Salazar L, Sandoval H, García-Pérez D, Klein-Zampaña CJ, Barbella-Aponte RA, Segura T and Serrano-Heras G: Proinflammatory macrophage secretome enhances temozolomide sensitivity in glioblastoma via pSTAT3-mediated downregulation of DNA repair enzymes. Sci Rep 15: 38875, 2025.
23. Li R, Shen Q, Wu N, He M, Liu N, Huang J, Lu B, Yao Q, Yang Y and Hu R: MiR-145 improves macrophage-mediated inflammation through targeting Arf6. Endocrine 60: 73-82, 2018.
24. Kishimoto T and Kang S: IL-6 revisited: From rheumatoid arthritis to CAR T cell therapy and COVID-19. Annu Rev Immunol 40: 323-348, 2022.
25. Favalli EG: Understanding the role of interleukin-6 (IL-6) in the joint and beyond: A comprehensive review of IL-6 inhibition for the management of rheumatoid arthritis. Rheumatol Ther 7: 473-516, 2020.
26. Pattanaik SS, Panda AK, Pati A, Padhi S, Tripathy R, Tripathy SR, Parida MK and Das BK: Role of interleukin-6 and interferon-α in systemic lupus erythematosus: A case-control study and meta-analysis. Lupus 31: 1094-1103, 2022.
27. Choy EH, De Benedetti F, Takeuchi T, Hashizume M, John MR and Kishimoto T: Translating IL-6 biology into effective treatments. Nat Rev Rheumatol 16: 335-345, 2020.
28. Mazzoni A, Salvati L, Maggi L, Capone M, Vanni A, Spinicci M, Mencarini J, Caporale R, Peruzzi B, Antonelli A, *et al*: Impaired immune cell cytotoxicity in severe COVID-19 is IL-6 dependent. J Clin Invest 130: 4694-4703, 2020.
29. Liu Y and Chen L: Impact of interleukin 6 levels on acute lung injury risk and disease severity in critically ill sepsis patients. World J Clin Cases 12: 5374-5381, 2024.
30. Sheridan R, Brennan K, Bazou D, O'Gorman P, Matallanas D and Mc Gee MM: Multiple myeloma derived extracellular vesicle uptake by monocyte cells stimulates IL-6 and MMP-9 secretion and promotes cancer cell migration and proliferation. Cancers (Basel) 16: 1011, 2024.
31. Hu Y, Chen H, Jin L, Chi X, Zhao J and Cao Q: Hypomethylation of IL6ST promotes development of endometriosis by activating JAK2/STAT3 signaling pathway. PLoS One 20: e0317569, 2025.
32. Aletaha D, Kerschbaumer A, Kastrati K, Dejaco C, Dougados M, McInnes IB, Sattar N, Stamm TA, Takeuchi T, Trauner M, *et al*: Consensus statement on blocking interleukin-6 receptor and interleukin-6 in inflammatory conditions: An update. Ann Rheum Dis 82: 773-787, 2023.
33. Hu J, Sun Y, Zuo X and Zou Y: Assessment of adverse events related to anti-interleukin-6 receptor monoclonal antibodies using the FDA adverse event reporting system: A real-world pharmacovigilance study. Expert Opin Drug Saf 23: 1327-1339, 2024.
34. Ambrosone A, Barbulova A, Cappetta E, Cillo F, De Palma M, Ruocco M and Pocsfalvi G: Plant extracellular vesicles: Current landscape and future directions. Plants (Basel) 12: 4141, 2023.
35. Xu Z, Xu Y, Zhang K, Liu Y, Liang Q, Thakur A, Liu W and Yan Y: Plant-derived extracellular vesicles (PDEVs) in nanomedicine for human disease and therapeutic modalities. J Nanobiotechnology 21: 114, 2023.
36. Li C, Zeng A, Li L and Zhao W: Emerging roles of plant-derived extracellular vesicles in biotherapeutics: Advances, applications, and future perspectives. Adv Biol (Weinh) 9: e2500008, 2025.
37. Emmanuela N, Muhammad DR, Iriawati, Wijaya CH, Ratnadewi YMD, Takemori H, Ana ID, Yuniati R, Handayani W, Wungu TDK, *et al*: Isolation of plant-derived exosome-like nanoparticles (PDENs) from *Solanum nigrum* L. berries and their effect on interleukin-6 expression as a potential anti-inflammatory agent. PLoS One 19: e0296259, 2024.
38. Song Y, Feng N, Yu Q, Li Y, Meng M, Yang X, Gan Z, Xu T, Tang C and Zhang Y: Exosomes in disease therapy: plant-derived exosome-like nanoparticles current status, challenges, and future prospects. Int J Nanomedicine 20: 10613-10644, 2025.
39. Chi J, Wang X, Song Y, Wang J, Guo L, Wang R and Wang G: Emerging plant-derived exosome-like particles reveal key therapeutic benefits. A comprehensive review of evidence. Int J Nanomedicine 20: 12393-12411, 2025.