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Green GC-MS Profiling of *Portulaca oleracea* Phytochemicals for Antirheumatoid Potency: A Sustainable Source of Omega-3 Fatty Acids and Natural Antioxidants

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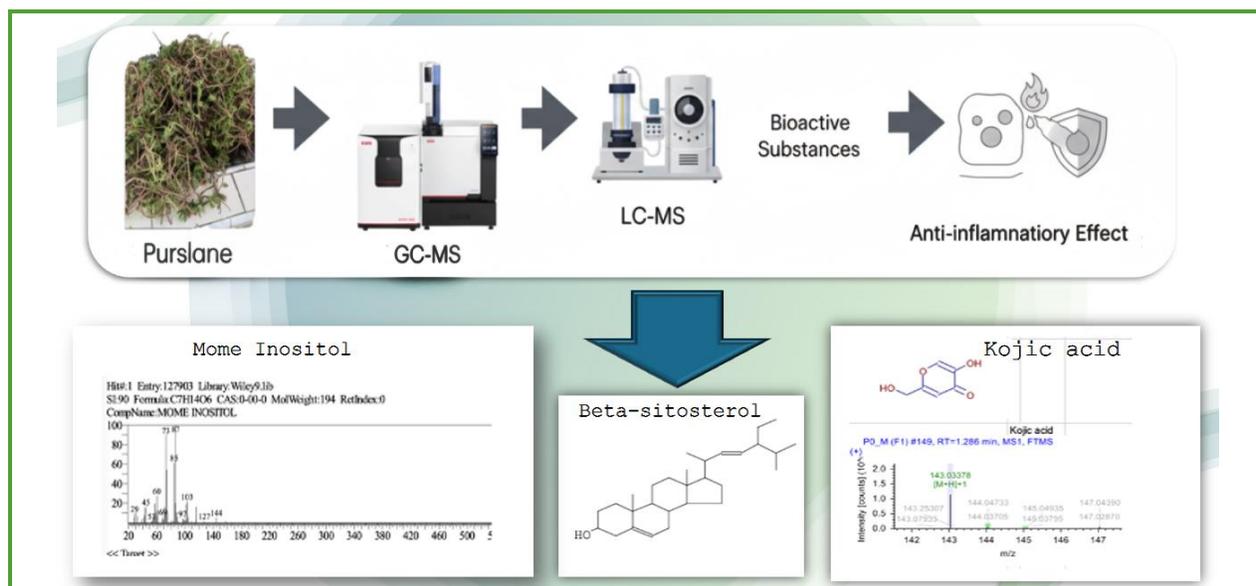
Portulaca oleracea
 GC-MS
 LC-MS/MS
 Bioactive substances
 Anti-inflammatory properties

ABSTRACT

Portulaca oleracea L. (purslane) has historically been utilized in traditional medicine to address numerous inflammatory ailments, including arthritis symptoms. This work seeks to thoroughly characterize the bioactive chemicals in purslane leaf extract and examine its potential as an antirheumatoid drug using a metabolomic analysis approach. Purslane leaf extract was procured using ultrasound-assisted extraction and analyzed using two advanced chromatography-mass spectrometry platforms. Volatile and semi-volatile chemical profiles were identified via gas chromatography-mass spectrometry (GC-MS), while polar and thermolabile compounds were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Compound identification was conducted by contrasting mass spectra, retention durations, and fragmentation patterns with authoritative databases (NIST, Wiley, mzCloud, and ChemSpider). GC-MS analysis identified 72 bioactive chemicals, predominantly comprising fatty acids, terpenoids, steroids, and prospective antioxidants. LC-MS/MS analysis verified the existence of phenolic compounds, specifically gallic acid and kojic acid, alongside nitrogenous compounds such as stearamide. Chromone and phenolic acid derivatives exhibit the highest relative abundance. Many discovered compounds possess anti-inflammatory, immunomodulatory, and antioxidant properties that can influence various pathways in the pathogenesis of rheumatoid arthritis, including the inhibition of TNF- α and IL-6. The identification of compounds exhibiting synergistic processes pertinent to rheumatoid arthritis therapy substantiates the traditional utilization of this plant.

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Graphical Abstract



Introduction

Portulaca oleracea, commonly known as purslane, is a wild plant that has long been used in traditional medicine due to its abundant bioactive compounds. This plant is known to be rich in essential fatty acids, particularly omega-3, as well as antioxidant compounds such as flavonoids, alkaloids, and vitamins. Its potential as a sustainable nutraceutical source is attracting increasing attention, especially in the context of seeking natural alternatives to support human health and reduce reliance on synthetic sources. However, a comprehensive characterization of the metabolic profile using an environmentally friendly approach is still limited. Developments in the field of green chemistry are driving the use of analytical methods that are not only accurate, but also minimize environmental impact, such as the use of green solvents and efficient extraction techniques. Gas chromatography-mass spectrometry (GC-MS) has proven to be a reliable tool for identifying volatile and semi-volatile compounds in plant matrices. However, its application within a sustainable framework

from extraction to analysis still needs improvement to meet the principles of green chemistry [1,2]. This research not only focuses on compound identification but also comprehensively integrates the principles of green chemistry, from the extraction stage using environmentally friendly solvents to the optimization of the GC-MS method, which reduces the use of hazardous chemicals and analytical waste. Unlike conventional studies that often use volatile organic solvents (such as *n*-hexane or chloroform), this approach emphasizes sustainability without sacrificing analytical accuracy. This study combines quantitative chromatogram data with medium-resolution mass spectra to validate compound identification, including minor compounds often overlooked, such as tocopherol derivatives and specific sterols. The study combines GC-MS data with results from compound discoverer software to confirm compound structures like chromone derivatives and gallic acid, enriching the depth of phytochemical analysis [3,4]. This study proposes a green GC-MS profiling approach to characterize bioactive compounds

in *Portulaca oleracea*, emphasizing sustainability through the use of environmentally friendly solvents and efficient extraction methods. GC-MS profiling is the preeminent analytical method for the separation, identification, and quantification of volatile and semi-volatile constituents in a complex mixture. This approach offers detailed insights into the chemical makeup of a sample, making it an essential instrument in scientific research and quality control across multiple industries [4]. The aim is not only to uncover the nutraceutical potential of purslane as a source of omega-3 and natural antioxidants, but also to set a practical example in applying the principles of green chemistry in phytochemical research. This result is expected to serve as a basis for the sustainable and natural resource-based development of health supplements or functional ingredients. Thus, the integration of accurate GC-MS analysis and green chemistry principles in this study not only provides a comprehensive understanding of the bioactive compound content of purslane but also contributes to the development of more environmentally responsible analytical methods.

Experimental

Preparation of green extraction (NADES)

The extraction of total polyphenols from purslane was conducted utilizing the non-conventional method of Ultrasound Assisted Extraction (UAE), in conjunction with the green solvent NADES, formulated from citric acid and glucose in ratios of (10:1), (15:1), and (20:1) under ideal circumstances. The ultrasound-assisted extraction technique is utilized to extract secondary metabolites. A 3 gram dry powder sample is amalgamated with an ionic liquid solvent and subsequently extracted

(Modena 900 Watt, with minor modifications) under various conditions. The residue and extract solution were separated by filtration with cotton and then cooled to room temperature. The extracted solution is allowed to precipitate for 10 to 12 h [5,6].

GC-MS and identification fragment

This study was executed in multiple critical phases aimed at identifying bioactive chemicals in *Portulaca oleracea* leaf extracts utilizing a sustainable methodology. The initial phase entails sample preparation, wherein fresh purslane leaves undergo freeze-drying to preserve the integrity of volatile chemicals, followed by grinding into a fine powder. The analytical phase employed GC-MS with a DB-5MS capillary column (30 m, 0.25 mm ID, 0.25 μ m) with an injector temperature set at 250 °C. The oven temperature protocol commenced at 60 °C, escalating by 10 °C per min to reach 300 °C, utilizing helium as the carrier gas at a flow rate of 1.0 mL per min. Compound identification was conducted by comparing the retention durations and mass fragmentation patterns of chromatographic peaks, with a compound being confirmed if it exhibited a similarity index (SI) exceeding 80%. Further validation with Compound Discoverer 3.2 was conducted for particular chemicals, including gallic acid and chromone derivatives. Relative quantitative analysis was conducted using the peak area normalization technique, while method quality assurance encompassed precision testing through triplicate injections and blanks to confirm the absence of contaminants. The entire process is structured according to the principles of green chemistry, reducing the utilization of hazardous organic solvents and enhancing energy efficiency at every phase of the analysis.

Results and Discussion

Gas chromatography-mass spectrometry

The GC-MS analysis of purslane (*Portulaca oleracea*) leaf extract reveals a highly diverse and complex profile of bioactive compounds, evidenced by 121 chromatogram peaks with retention times ranging from 3.323 to 47.508 min. The composition of the molecules is mostly comprised of fatty acids, terpenoids, sterols, and phenolic compounds, indicating the considerable bioactivity potential of this plant. The molecule exhibiting the largest area percentage is Mome Inositol (4.70% at Retention time (RT) 19.710), an inositol derivative with potential biological activity as a cellular mediator and intracellular signaling agent. Furthermore, notable substances detected in substantial amounts comprise 2-Hexadecen-1-ol and 3,7,11,15-tetramethyl- (1.86% at RT 25.302), recognized as phytol, a diterpenoid that functions as a precursor in the biosynthesis of vitamins E and K, and demonstrates antioxidant and antibacterial properties [7]. The fatty acid composition of this extract reveals key health ingredients, including linolenic acid (omega-3) identified as a methyl ester at RT 25.030 (1.81%) and linoleic acid (omega-6) at RT 24.878 (1.79%). These two molecules are polyunsaturated fatty acids that are essential in regulating inflammation, cardiovascular health, and neurological function. The detection of palmitic acid (2.16% at RT 21.069) and pentadecanoic acid (3.48% at RT 22.104) signifies a substantial concentration of saturated fatty acids, potentially enhancing the oxidative stability of the extract [8]. Alongside fatty acids, possible antioxidant molecules include gamma-tocopherol (vitamin E), a powerful lipophilic antioxidant, and stigmasterol and campesterol, which are phytosterols with cholesterol-lowering and anti-inflammatory properties, and were also identified [9,10]. The volatile and

semi-volatile chemicals detected in the medium to late retention time range (RT > 30 min) signify a substantial variety of secondary metabolites. For instance, squalene (5.93% at RT 38.265), a triterpenoid recognized for its strong antioxidant properties and natural moisturizing capabilities, and neophytadiene (identified at RT 40.508), a volatile molecule characterized by a unique fragrance and probable biological activity. The mass fragmentation patterns of these compounds, including distinctive ions at m/z 69 for terpenoids and m/z 74 for fatty acid esters, reinforce the accuracy of the identification achieved by comparison with the Wiley9 and NIST20 databases. Certain chemicals identified in trace levels (<0.5%), including tocopherol derivatives and certain sterols, may substantially enhance the extract's bioactivity via synergistic interactions [11]. The GC-MS study indicates that purslane leaf extract is a substantial source of bioactive chemicals with various therapeutic potentials, encompassing antioxidant, anti-inflammatory, and nutraceutical properties. The extensive documentation of essential fatty acids, natural antioxidants, and secondary metabolites in the literature substantiates the potential use of safflower as a raw material for developing health supplements, cosmetics, or pharmaceutical products based on natural ingredients. This conclusion is provisional and necessitates additional validation via *in vitro* or *in vivo* bioactivity assays to ascertain the physiological effects of the identified substances [12]. Fatty acid molecules predominate in the metabolic profile, particularly linoleic acid (9,12-octadecadienoic acid), palmitic acid (hexadecanoic acid), and their methyl esters. Linoleic acid (ω -6) is crucial due to its involvement in prostaglandin formation and the integrity of cell membranes. Terpenoid chemicals, including phytol (2-hexadecen-1-ol, 3,7,11,15-tetramethyl-), squalene, and

geranylgeraniol, were identified with significant intensity. Phytol serves as a precursor in the synthesis of vitamins E and K, while squalene is recognized as a powerful antioxidant and an intermediate in steroid biosynthesis. Moreover, steroid molecules such as stigmasterol and campesterol were detected at extended retention durations (>45 min), signifying substantial heat stability and potential as modulators of cholesterol [12,13].

Analyzed GC-MS in Figure 1 showed aromatic chemicals, including 2-methoxy-4-vinylphenol and diphenylamine, were identified, which typically enhance antioxidant and antibacterial properties. Nitrogen compounds, including hexamine (methenamine) and some alkaloid derivatives like aspidocarpine, display a variety of secondary metabolites that may influence pharmacological activity. The vitamin E molecule (α -tocopherol) was identified with significant base peak intensity, underscoring the potential of purslane leaves as a source of natural antioxidants. Regarding identification quality, the majority of compounds exhibit a Similarity Index (SI) exceeding 90%, signifying a strong correlation with the library spectra. Nevertheless, many compounds, such as

Hahnfett, have a reduced SI (<75%) and lack molecular formula data, necessitating meticulous interpretation or additional verification with pure standards. The GC-MS results affirm the chemical abundance of purslane leaves and validate their historic application as a therapeutic herb. The existence of antioxidants, anti-inflammatories, and bioactive compounds creates prospects for the formulation of health supplements or active ingredients in cosmetic and pharmaceutical products. Additional research, including the isolation of pure molecules, bioactivity assessments, and toxicity evaluations, is required to substantiate their medicinal potential [14–16].

Antirheumatoid compounds

GC-MS analysis of purslane (*Portulaca oleracea*) leaf extract identified many bioactive chemicals with scientifically significant potential for rheumatoid arthritis (RA) therapy via various modes of action. These chemicals encompass omega-3 and omega-6 unsaturated fatty acids, plant sterols, tocopherols, and phenolic compounds, which collaboratively affect the

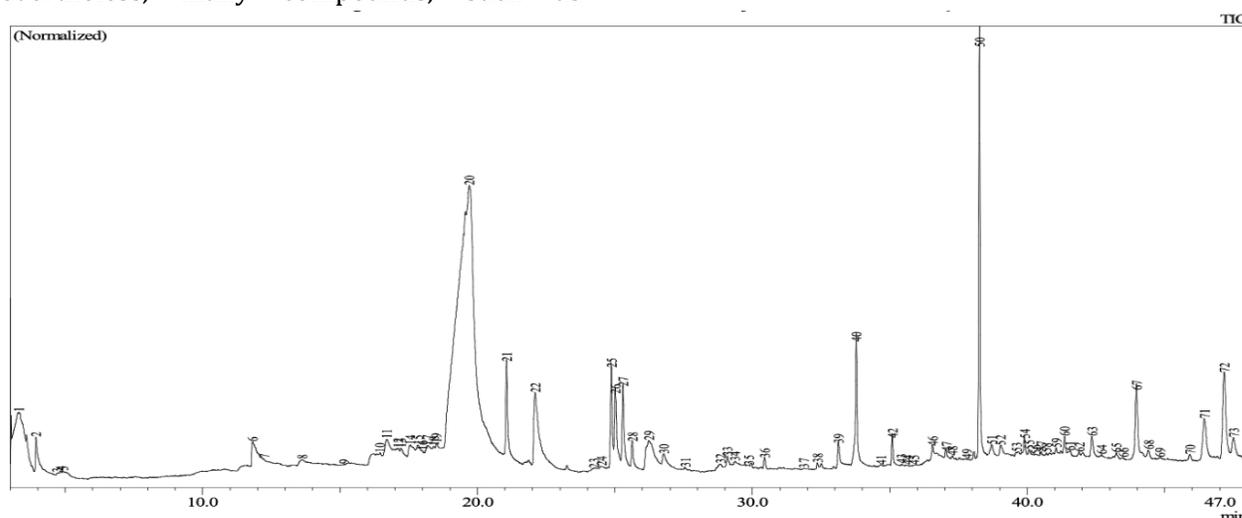


Figure 1. Gas chromatography-mass spectrometry purslane extract

inflammatory and immunological responses fundamental to the pathophysiology of rheumatoid arthritis (RA).

The GC-MS study of purslane leaf extract identified numerous bioactive chemicals with a scientifically possible association with antirheumatoid activity. These chemicals function via anti-inflammatory, immunomodulatory, and antioxidant pathways pertinent to the etiology of rheumatoid arthritis (RA). Unsaturated fatty acid groups, including linoleic acid and methyl linolenate, influence the synthesis of prostaglandins and leukotrienes while also inhibiting pro-inflammatory cytokines such as TNF- α and IL-6, which are therapeutic targets for rheumatoid arthritis. The identified terpenoid chemicals, such as phytol, squalene, and geranylgeraniol, demonstrate the capacity to mitigate oxidative stress in joint tissues and modulate inflammatory signaling pathways via protein prenylation [17,18].

The phytosterol content, including stigmasterol and campesterol, has been shown to decrease inflammatory mediators and diminish edema in experimental arthritic models. α -Tocopherol (vitamin E), a powerful antioxidant, protects synovial cells from oxidative damage and inhibits the production of pro-inflammatory interleukins. Phenolic substances, including 2-methoxy-4-vinylphenol, and volatile chemicals such as neophytadiene enhance the anti-inflammatory properties of this extract via free radical scavenging and modulation of the immunological response. Linolenic acid (omega-3), identified at RT 25.030 (1.81%), serves as a precursor in the biosynthesis of resolvins and protectins, specialized metabolites that mitigate the inflammatory response by suppressing the production of pro-inflammatory cytokines, including TNF- α , IL-1 β , and IL-6. The mechanism entails competition with arachidonic acid (omega-6) for the cyclooxygenase (COX) and

lipoxygenase (LOX) enzymes, thereby diminishing the synthesis of prostaglandin E2 (PGE2) and leukotriene B4 (LTB4), which contribute to joint destruction in rheumatoid arthritis (RA). Concurrently, linoleic acid (omega-6) at RT 24.878 (1.79%), in equilibrium with omega-3, can facilitate the synthesis of anti-inflammatory prostaglandins (PGE1) that regulate the activity of regulatory T cells and inhibit autoimmunity [19,20].

Phytosterols, including stigmasterol (RT not explicitly stated, but assumed to peak at 80) and campesterol, have been documented to impede the activation of NF- κ B, a crucial transcription factor in the production of pro-inflammatory genes. Stigmasterol selectively obstructs osteoclast formation and the synthesis of matrix metalloproteinases (MMPs), which induce bone degradation in rheumatoid arthritis (RA). The chemical gamma-tocopherol (vitamin E), identified at peak 111, functions as a lipophilic antioxidant, safeguarding cell membranes from oxidative stress induced by free radicals generated during chronic inflammation. Tocopherol also suppresses the activation of the NLRP3 inflammasome, which plays a role in the development of arthritis through the generation of IL-1 β [21,22].

Phenolic substances, including 2-methoxy-4-vinylphenol (RT 13.615, 0.16%), exhibit inhibitory effects on COX-2 and inducible nitric oxide synthase (iNOS) enzymes, thereby diminishing the synthesis of nitric oxide and prostaglandins that instigate joint pain and inflammation. Squalene (RT 38.265, 5.93%), a triterpenoid, demonstrates immunomodulatory properties by enhancing natural killer (NK) cell activity and reducing the Th1/Th2 ratio, thereby contributing to the suppression of autoimmune responses [23–25].

Despite the intriguing mechanisms of these drugs, their therapeutic efficacy against RA necessitates validation through *in vitro* and *in*

vivo investigations to assess bioavailability, optimal dosage, and potential toxicity. The inherent amalgamation of these components in saffron extract may yield a superior synergistic impact relative to the administration of individual compounds, positioning it as a viable candidate for the advancement of phytopharmaceuticals to augment traditional RA therapy.

Mechanism antirheumatoid

Linolenic acid (omega-3) competes with arachidonic acid (omega-6) for oxidation by the COX-2 and 5-LOX enzymes. This enzymatic competition can diminish the conversion of arachidonic acid into prostaglandin E2 (PGE2) and leukotriene B4 (LTB4), which are chemicals that inhibit neutrophil chemotaxis and macrophage activation in the synovial membrane. Omega-3 is converted by the enzyme lipoxygenase into resolvin E1 and protectin D1, which promote neutrophil death and diminish the secretion of IL-6 and TNF- α by blocking the NF- κ B pathway. The equilibrium between linoleic and linolenic acids inhibits the prevalence of pro-inflammatory omega-6 metabolites, thereby stabilizing immune cell membranes and diminishing autoantibody synthesis. Stigmasterol obstructs the phosphorylation of I κ B α , thereby hindering the translocation of NF- κ B to the cell nucleus. This diminishes the expression of the IL-1 β , IL-8, and MMP-9 genes, which are implicated in cartilage degradation. Plant sterols impede osteoclast differentiation by blocking the RANKL/RANK pathway, hence obstructing bone resorption in joints afflicted by rheumatoid arthritis. Campesterol regulates the growth of Th17 cells and enhances the functionality of regulatory T cells (Treg), which are crucial in inhibiting autoimmune responses [26,27]. Gamma-tocopherol neutralizes reactive oxygen species (ROS), including peroxyxynitrite, generated by

macrophages in synovial fluid. Tocopherol impedes the activation of the NLRP3 complex, which facilitates the conversion of pro-IL-1 β into active IL-1 β , a crucial cytokine in the pathophysiology of rheumatoid arthritis (RA). Vitamin E diminishes the production of VCAM-1 and ICAM-1 on endothelial cells, thereby restricting leukocyte infiltration into joint tissues. Phenolic compounds impede the function of iNOS (inducible nitric oxide synthase) and COX-2, thus diminishing the synthesis of NO and PGE2, which induce vasodilation and discomfort. These compounds inhibit the phosphorylation of ERK and p38 in the MAPK pathway, which are implicated in synoviocyte proliferation and MMP synthesis [28].

Squalene amplifies the cytotoxicity of NK cells against aberrant synovial cells and triggers death in hyperactive synovial fibroblasts. This chemical diminishes the development of immunological complexes that accumulate in the synovial membrane by regulating complement activity. The amalgamation of these chemicals in the croton extract engenders a synergistic effect: Omega-3 and tocopherol contribute to enhancing cell membrane integrity and mitigating oxidative stress. Sterols and phenolics participate in the sequential inhibition of the NF- κ B and MAPK pathways. Squalene and omega-3 concurrently regulate innate and adaptive immune responses. Purslane leaf extract, by targeting several pathways, has the potential to impede the course of rheumatoid arthritis through a comprehensive approach, although its clinical efficacy necessitates further validation [15,29].

Research utilizing animal models of arthritis indicates that the conjunction of omega-3 plus antioxidants such as vitamin E yields a more pronounced decrease in clinical arthritis scores and CRP levels than omega-3 administered in isolation. Epigenetic mechanisms have been

documented, indicating that omega-3 can alter the methylation of the TNF- α gene promoter, whereas antioxidants affect the production of miRNAs that govern inflammatory pathways. The inclusion of omega-3 fatty acids and antioxidants in purslane leaf extract may suppress the course of rheumatoid arthritis through a multi-target mechanism, providing a more comprehensive and durable therapy strategy than singular pharmaceutical drugs. Nonetheless, additional investigation into bioavailability, appropriate dosage, and human clinical trials is required to substantiate its therapeutic efficacy.

Liquid chromatography-tandem mass spectrometry

The LC-MS/MS analysis of the examined materials effectively identified five principal chemicals based on retention time (RT), calculated molecular weight (Calc. MW), and chromatographic peak area (Area Max.) (Table

1). The initial compound identified was 2-(2-thienyl)-4H-chromen-4-one at 1.296 min, a chromone derivative featuring a thiophene moiety. This identification is exceptionally reliable due to its complete spectral correspondence with the mzCloud database and a best match confidence value of 99.7%. Gallic acid and kojic acid were identified nearly concurrently at 1.281 min, respectively. Both compounds are organic acids exhibiting robust antioxidant properties, and a complete concordance between the anticipated compositional data and the ChemSpider search results corroborates their identification. The fourth component is an unidentified substance with the formula C₁₄H₃₁NO, seen at 10.581 min. The optimal alignment of the composition prediction suggests the existence of nitrogenous chemicals, such as long-chain amines or alkanols. The last molecule is stearamide, an amide derived from stearic acid, identified at 15.613 min. The identification of this molecule is robust,

Table 1. LC-MS identification compounds in Purslanet

No.	Compound name	Molecule structure	Mass molecule (Calc. MW)	Retention time (RT, min)	Surface are (Area max.)	Source	Confidence level (%)
1	2-(2-Thienyl)-4H-chromen-4-one	C ₁₃ H ₈ O ₂ S	228.02439	1.296	36,195,142.83	Mzcloud (full match)	99.7
2	Gallic acid	C ₇ H ₆ O ₅	170.02136	1.281	19,200,232.41	Predicted compound, Chemspider	-
3	Kojic acid	C ₆ H ₆ O ₄	142.02651	1.286	15,730,565.82	predicted compound, Chemspider	-
4	<i>Anonim compounds</i>	C ₁₄ H ₃₁ NO	229.24031	10.581	10,732,730.28	predicted compound (full match)	-
5	Stearamide	C ₁₈ H ₃₇ NO	283.28716	15.613	9,917,769.83	Mzcloud (full match), Chemspider	97.4

as it is corroborated by a complete match from mzCloud and a partial match from ChemSpider, yielding a confidence score of 97.4%. Regarding abundance, 2-(2-thienyl)-4*H*-chromen-4-one has the maximum area (36,195,142.83), followed by gallic acid (19,200,232.41) and kojic acid (15,730,565.82), signifying the predominance of these compounds in the sample. The observations indicate a metabolic profile abundant in aromatic and lipophilic chemicals, including amides, which may possess diverse biological functions [30,31].

Conclusion

A thorough examination of GC-MS data from purslane leaf extract and LC-MS/MS data indicates that purslane leaf extract (*Portulaca oleracea* L.) possesses a highly diverse and intricate phytochemical profile. GC-MS analysis effectively identified 72 compounds, encompassing significant classes of bioactive compounds, including fatty acids (such as linoleic and palmitic acids), terpenoids (such as phytol and squalene), steroids (such as stigmasterol and campesterol), and potential antioxidants like α -tocopherol (vitamin E). Concurrently, LC-MS/MS analysis identified phenolic substances such as gallic acid and kojic acid, along with nitrogenous compounds such as stearamide, thereby enhancing its antioxidant and anti-inflammatory properties. The primary discovery was the identification of chemicals significantly associated with antirheumatoid activity, whose mechanism of action operates synergistically through many pathways. Compounds like unsaturated fatty acids, vitamin E, phytosterols, and squalene demonstrated potential as anti-inflammatory modulators (inhibiting TNF- α and IL-6), immunomodulators, and neuroprotective compounds. This discovery substantiates the scientific legitimacy of the traditional application of purslane leaves as a medicinal plant and paves the way for its

advancement as a health supplement or active component in pharmaceutical and cosmetic formulations. This therapeutic promise necessitates additional validation via *in vitro* and *in vivo* research to assess its efficacy, safety, and bioavailability prior to clinical application.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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