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Phytochemical Screening, Antioxidant and Antibacterial Activity of Martynia annua L. Leaf Extracts

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Abstract

In the quest for natural antioxidants and antibacterial chemicals to fight oxidative stress and antimicrobial resistance, medicinal plants continue to be an essential source of innovative therapeutic agents. The goal of the current research was to assess the antibacterial potential, antioxidant activity, and phytochemical components of various solvent extracts of Martynia annua L. leaves. Petroleum ether, ethanol, and distilled water were used in cold maceration to create leaf extracts. Alkaloids, flavonoids, phenolics, tannins, glycosides, terpenoids, steroids, saponins, carbohydrates, and proteins were found in preliminary phytochemical screening; the ethanolic extract showed the widest range of secondary metabolites. The ethanol extract had the greatest total phenolic content (112.45 ± 3.28 mg GAE/g) and total flavonoid content (68.74 ± 2.01 mg QE/g), according to quantitative estimate. The DPPH free-radical scavenging test was used to measure antioxidant activity. The ethanolic extract showed a dose-dependent response with the lowest IC•₀ value of 45.23 µg/mL, while the aqueous extract had a value of 74.85 µg/mL. When Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa were screened for antibacterial activity using agar well diffusion and broth microdilution, the ethanol extract demonstrated the strongest activity, with the largest zone of inhibition (18.14 ± 0.68 mm) and the lowest minimum inhibitory concentration (0.625 mg/mL) against B. subtilis. There was very little action in the petroleum ether extract. These findings confirm the traditional therapeutic usage of M. annua and call for further separation and characterisation of its bioactive components. They also show that the ethanolic leaf extract of M. annua is a potential source of antioxidant and antibacterial phytochemicals.

Keywords: Martynia annua, leaf extract, phytochemical screening, antioxidant activity, antibacterial activity.

1. INTRODUCTION

1.1 General Background

Plants have been the cornerstone of medicinal systems since antiquity, and their enduring importance in world healthcare is indisputable. Up to 80% of people in underdeveloped nations rely on plant-based traditional medicine for basic healthcare, as the World Health Organization (WHO) has long acknowledged [1]. This long-lasting dependence is deeply ingrained in the

unmatched chemical richness and biological power of the plant world, rather than just being a question of cultural custom or financial accessibility. The enormous reservoir of pharmacological leads that nature offers is shown by iconic medicines like morphine from Papaver somniferum, quinine from Cinchona bark, the antimalarial artemisinin from Artemisia annua, and the anticancer agent taxol from Taxus brevifolia [2]. A serious scientific effort to establish the empirical knowledge of traditional medicine is motivated by the holistic and often synergistic character of herbal combinations, which provide a beneficial supplement to single-molecule synthetic medications [2, 3].

The ability of medicinal plants to biosynthesize a wide range of specialized chemicals known as secondary metabolites is largely responsible for their therapeutic value. Secondary metabolites serve as ecological mediators, protecting the plant from herbivory, microbial infection, and UV radiation, or promoting pollination and seed dispersion, in contrast to primary metabolites, which are necessary for basic cellular functions [3]. These substances constitute a pre-evolved library of bioactive chemicals with exceptional target selectivity when viewed from a pharmacological perspective. Alkaloids, flavonoids, tannins, terpenoids, saponins, and a wide range of phenolic compounds are major classes of these phytoconstituents. These groups exhibit a broad range of biological activities; for example, flavonoids and other phenolics are praised for their strong antioxidant properties, which are based on their capacity to scavenge free radicals and chelate pro-oxidant metal ions, while many alkaloids have significant effects on the central nervous system [3,4]. The identification of new therapeutic agents from conventional treatments is based on the scientific study of these metabolites, which is essential to the discipline of pharmacognosy [5].

Driven by the confluence of two major global health issues, the quest for natural antioxidant and antibacterial medicines has become a vital research priority within this area. The first is the widespread involvement of oxidative stress in the development and course of degenerative and chronic illnesses. Oxidative stress is a situation in which the production of free radicals and reactive oxygen species (ROS) exceeds the body's natural antioxidant defense mechanisms, causing oxidative damage to proteins, lipids, and DNA [6]. A wide range of illnesses, including cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases like Parkinson's and Alzheimer's, and general aging processes, are now closely linked to this molecular chaos [6, 7]. The exogenous supply of antioxidants from dietary and botanical sources is essential for preserving redox equilibrium and reducing the risk of illness, even though the human body has complex endogenous antioxidant processes. As a result, the protective properties of plants high in phenolic and flavonoid chemicals are being thoroughly investigated [4,7].

The second issue is the unrelenting rise of antimicrobial resistance (AMR) throughout the world, which poses a danger to contemporary medicine by bringing it back to the days before antibiotics. Methicillin-resistant Staphylococcus aureus (MRSA) and extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae are examples of multidrug-resistant (MDR) pathogens that have emerged as a result of the excessive and frequently inappropriate use of conventional antibiotics [8]. Novel antibacterial drugs with distinct modes of action are desperately needed since these resistance infections are linked to increased morbidity,

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mortality, and healthcare expenses [8,9]. Medicinal plants provide a rational and very promising line of inquiry in this regard [3,9]. Compared to single-target synthetic medicines, plant extracts have the capacity to act on several bacterial sites at once because they contain complex combinations of secondary metabolites. This method not only increases antibacterial efficacy but also greatly lowers the likelihood of resistance development. Therefore, a crucial need for creating safe, efficient, and long-lasting therapeutic options to address these two global concerns is the systematic bioprospecting of medicinal flora for their antioxidant and antibacterial capabilities [10].

1.2 About the Plant

Figure 1.1 Photograph of Martynia annua plant and leaves

The only member of the monogeneric family Martyniaceae, Martynia annua L., is an interesting plant that falls in between a common weed and a treasured part of many ancient pharmacopoeias. The family Martyniaceae, which belongs to the Lamiales order, is distinguished by its viscid, glandular pubescence and characteristic horned fruits [11]. The plant's distinctive fruit form is vividly captured by a variety of descriptive common names that are used worldwide. Devil's Claw, Tiger's Claw, and Ice Plant are the most common English nicknames. It is known by equally evocative vernacular names in the vast ethnolinguistic landscape of the Indian subcontinent: Bichhu or Bagh-nakha (Hindi), Puli-nakham (Telugu), Kaakkay-shengaai (Tamil), and Hata-jodi (Sanskrit), all of which refer to the fruit's resemblance to a pair of joined hands or a sharp claw [11,12].

According to botany, M. annua is an upright, annual plant that usually reaches a height of 0.3 to 1.2 meters. Its whole surface is coated in a thick covering of glandular hairs that give it a

sticky texture. Often characterized as slightly succulent, the leaves are simple, opposite, decussate, and have a broadly ovate to deltoid form with cordate bases and irregularly dentate edges [11,12]. Large, eye-catching, zygomorphic blooms are produced by the inflorescence, a terminal raceme. The flower has an exotic, orchid-like look thanks to the corolla, a tubular structure with five flared lobes that varies in color from light pink to white and is often ornamented with a dramatic purple or yellowish nectar tube in the neck. The fruit, an initially fleshy, drupaceous capsule that reveals a hard, woody endocarp upon drying and dehiscence, is the most distinctive and titular characteristic of the plant. Each of the two boat-shaped mericarps that result from this endocarp's split ends in two pointed, curving, divergent horns. This structure, which hooks onto the hair and skin of passing animals, is a traditional adaption for epizoochorous seed dispersion [11,13].

M. annua is found in tropical and subtropical areas of the globe. The plant, which is thought to have originated in Mexico, Central America, and the Caribbean, has undergone widespread naturalization worldwide, perhaps because to its efficient seed-dispersal mechanism [11]. on tropical Africa, Southeast Asia, and the Indian subcontinent, it is now often seen on wastelands, roadsides, and disturbed habitats. It is found across the plains and at moderate altitudes in India, and it does especially well in the semi-arid areas after the arrival of the monsoon [11,12].

A significant amount of ethno-pharmacological information is derived from the plant's deep and varied traditional therapeutic significance. Almost every part of the plant has been used medicinally, including the roots, leaves, fruits, and seeds. Traditionally, a paste made from the leaves is used topically to heal burns, wounds, and localized inflammations as well as to relieve itching caused by insect stings and bites [11,13]. Additionally, certain tribal cultures utilize the leaves as a gargle for sore throats and as an anticonvulsant medication to treat epilepsy, a practice that has attracted a lot of scientific attention [14]. In addition to the fruits' traditional use as an anti-inflammatory, a paste made from them is sometimes used to treat alopecia, which is thought to promote hair growth. The root is said to have analgesic and antirheumatic qualities, while the oil derived from the seeds is used to treat skin conditions [13,14]. A strong ethnopharmacological foundation for its methodical scientific examination is provided by the documented pharmacological properties, which include anti-epileptic, wound-healing, analgesic, and anti-inflammatory effects [15].

1.3 Research Problem

There is a substantial gap in the thorough, comparative analysis of Martynia annua L.'s biological activities as a function of extraction solvent, despite the plant's widespread traditional use and the promising range of documented pharmacological properties. The polarity of the solvent employed during extraction has a significant impact on the phytochemical content and, hence, the bioactivity of a plant extract [16]. Different kinds of secondary metabolites, such as polar phenolic compounds, moderately polar flavonoids, and non-polar terpenoids, would be differently soluble in solvents such water, methanol, ethanol, ethyl acetate, and chloroform. A comprehensive, side-by-side comparison of the aerial parts employing a spectrum of solvents of increasing polarity is not easily accessible, although some

first investigations documenting the antibacterial or antioxidant capabilities of specific extracts of M. annua leaves [15,17]. In order to determine the best extraction method for separating bioactive principles and to demonstrate a strong relationship between the solvent-specific phytochemical profile and the observed antioxidant and antibacterial potencies, such a head-to-head comparison is crucial [18]. To close this gap and provide precise, fact-based information on the relative effectiveness of various leaf extracts, a thorough experimental review is desperately required.

1.4 Aim

The aim of the present study is to evaluate the phytochemical constituents, antioxidant activity, and antibacterial potential of different solvent extracts derived from the aerial parts of the leaves of Martynia annua L.

2. OBJECTIVES PF THE STUDY

To achieve the stated aim, the following specific objectives were set:

1. To prepare different solvent extracts (aqueous, methanolic, ethanolic, ethyl acetate, and chloroform) of Martynia annua leaves.
2. To calculate the percentage yield of each successive solvent extract.
3. To perform a preliminary phytochemical screening of all extracts to identify the major classes of secondary metabolites present [19].
4. To estimate the total phenolic content of each extract spectrophotometrically using the Folin-Ciocalteu method [20].
5. To estimate the total flavonoid content of each extract spectrophotometrically using the aluminium chloride colorimetric method [20].
6. To evaluate the in vitro antioxidant activity of the extracts using standard free radical scavenging assays, such as the DPPH and FRAP assays [7].
7. To evaluate the in vitro antibacterial activity of the extracts against selected Gram-positive and Gram-negative bacterial strains using the agar well diffusion method [10].
8. To compare the biological activities of the different extracts and correlate them with their phytochemical profiles in order to identify the most potent solvent system for extracting bioactive molecules from the plant.

3. MATERIALS AND METHODS

3.1 Study Design

The current study was planned as an experimental, lab-based research project. To guarantee accuracy and repeatability, every experiment was conducted in a controlled laboratory setting. Over the course of six months, from January 2024 to June 2024, the research was carried out at the Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Sciences, RKDF, University, Bhopal. The same institutional facilities were used for all bench work, including extraction, phytochemical screening, estimate of total phenolic and flavonoid contents, antioxidant tests, and antibacterial assessment.

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3.2 Plant Collection

The medicinal plant garden and adjacent uncultivated portions of the RKDF University campus in Bhopal, M.P., India, provided the fresh aerial parts, which included mature and healthy Martynia annua L. leaves. The gathering took place in August 2023, when the plant was at its height of vegetative development after the monsoon rains. Only leaves that were completely developed, green, and free of disease were chosen. The gathered plant material was promptly sent to the lab for further processing after being put in sterile, labeled polythene bags. Dust, dirt, and alien plant debris were all hand removed.

3.3 Plant Identification and Authentication

Taxonomic identification and authentication were performed on the gathered plant specimen. Professor Deepak Vyas, a certified plant taxonomist in the Department of Botany at Dr. Harisingh Gour University, Sagar, received fresh, undamaged leaves as well as blooming and fruiting parts. The specimen was carefully inspected using herbarium data and normal regional floras. The botanist provided an authenticity certificate after verifying that it was Martynia annua L. For future reference, a voucher specimen of the plant with the voucher specimen number M. annua/Herb/2024/001 was placed in the institutional herbarium.

3.4 Preparation of Leaf Powder

In order to remove any surface dust, dirt particles, and any microbiological pollutants, the verified, healthy leaves were first properly cleaned under mild running tap water. A final rinse with distilled water came next. To avoid photodegradation of light-sensitive phytoconstituents, the cleaned leaves were equally spread out on clean blotting paper and shade-dried in a dust-free, well-ventilated room away from direct sunshine. The leaves were dried for 15 days until they were crisp and brittle. A mechanical laboratory grinder was then used to ground the fully dried leaves into a coarse powder. To achieve a consistent particle size, the powdered material was run through a 40-mesh stainless-steel sieve. Before being used, the resultant fine leaf powder was weighed, put into an airtight, amber-colored glass container, and kept dry and cold, away from heat and moisture.

3.5 Preparation of Extracts

Using a sequential solvent extraction method with three solvents of increasing polarity—petroleum ether, ethanol, and distilled water—the cold maceration technique was used to extract bioactive chemicals from the powdered leaf material. To provide methodological consistency and enable comparative study, the same extraction technique was used for every solvent.

A 1000 mL Erlenmeyer flask was filled with 100 g of the dried leaf powder that had been carefully weighed. A 1:5 (w/v) plant material-to-solvent ratio was achieved by adding 500 mL of the corresponding solvent (petroleum ether, 95% ethanol, or distilled water) to the flask. To safeguard light-sensitive components, the flask was covered in aluminum foil and carefully sealed with a glass stopper. To enable effective mass transfer, the mixture was left to macerate at room temperature for 72 hours while being manually shaken every 12 hours. Each extract was first filtered through a clean muslin cloth to remove the bulk marc after the maceration

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time, and then it was vacuum-filtered using Whatman No. 1 filter paper to produce a clear filtrate. The aqueous extract was obtained by further extracting the marc that remained after the ethanol extraction using distilled water. To avoid heat destruction of the phytoconstituents, the resultant filtrates were concentrated separately under decreased pressure using a rotating vacuum evaporator at a temperature not to exceed 40°C. The solvent-free crude extracts were produced by further drying the semi-solid residues in a desiccator. After weighing the dried extracts, the following formula was used to determine each extract's % yield.

Extractive Yield (%) = (Weight of dried extract / Weight of leaf powder taken) × 100

Before being put through phytochemical and biological tests, the dried extracts were moved to sterile, airtight screw-capped vials and kept in a refrigerator at 4°C.

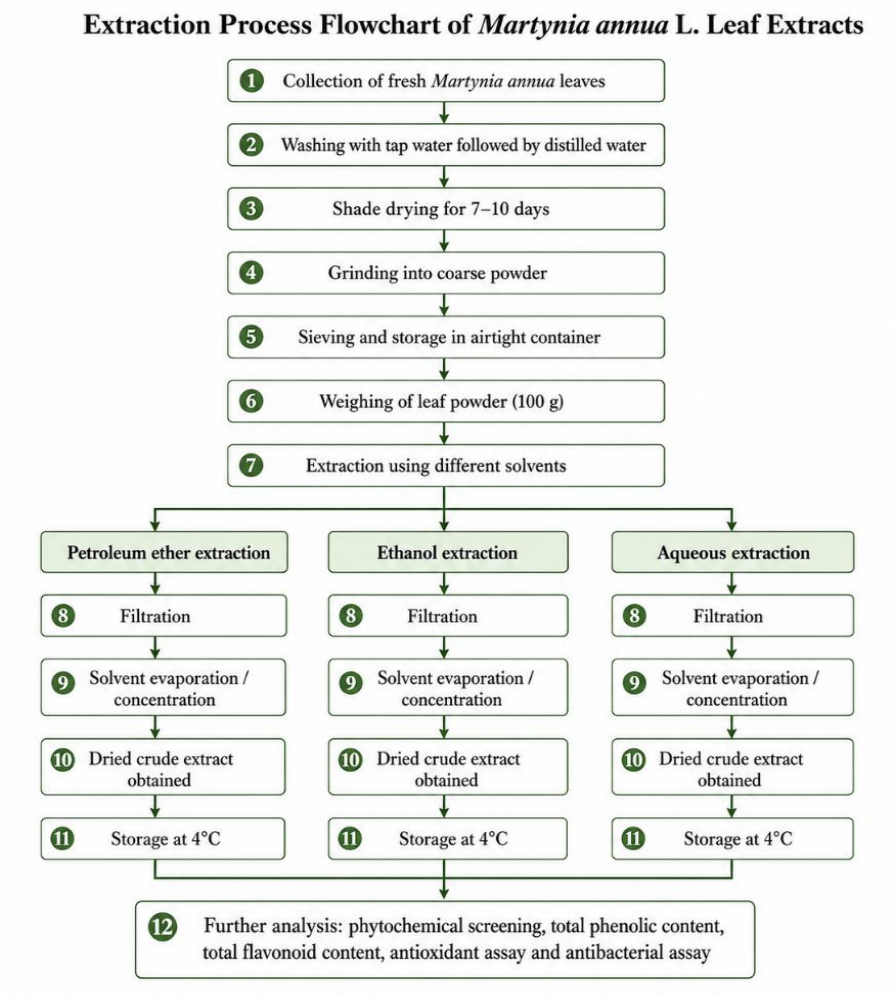

Figure 1 Schematic flowchart of extraction process for Marynia Annua Leaf Extracts

3.6 Preliminary Phytochemical Screening

All three prepared extracts (petroleum ether, ethanol, and aqueous) were subjected to qualitative phytochemical examination to determine if the main types of secondary metabolites are present or not. Standard, proven colorimetric and precipitation tests were carried out in accordance with the procedures outlined in phytochemistry manuals and standard

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pharmacopoeial textbooks. For the screening processes, the extracts were reconstituted at a concentration of 10 mg/mL in their corresponding solvents. The following are the particular tests carried out and the phytoconstituents they identify:

- Alkaloids: Mayer's test (cream precipitate), Wagner's test (reddish-brown precipitate), and Dragendorff's test (orange precipitate).
- Flavonoids: Shinoda test (pink to crimson colour with magnesium turnings and concentrated hydrochloric acid) and alkaline reagent test (intense yellow colour turning colourless on acidification).
- Phenolic compounds: Ferric chloride test (blue-black or greenish colour with 5% $FeCl_3$ solution).
- Tannins: Gelatin test (white precipitate with 1% gelatin solution containing 10% sodium chloride).
- Saponins: Froth test (persistent honeycomb-like froth on vigorous shaking with water).
- Glycosides: Keller-Kiliani test (reddish-brown ring at the interface on addition of glacial acetic acid, ferric chloride, and concentrated sulphuric acid).
- Terpenoids: Salkowski test (reddish-brown colour at the interface with chloroform and concentrated sulphuric acid).
- Steroids: Liebermann-Burchard test (greenish-blue colour with acetic anhydride and concentrated sulphuric acid).
- Carbohydrates: Molisch's test (violet ring at the interface with α-naphthol and concentrated sulphuric acid) and Fehling's test (brick-red precipitate on heating).
- Proteins: Biuret test (violet colour with sodium hydroxide and copper sulphate solution).

The results of each qualitative test were interpreted and semi-quantitatively recorded using the following notation system: absent (−), weakly present (+), moderately present (++), and strongly present (+++).

3.7 Total Phenolic Content

Using gallic acid as the reference standard, the Folin-Ciocalteu (F-C) colorimetric technique was used to spectrophotometrically estimate the total phenolic content (TPC) of the ethanolic and aqueous extracts. Because the test was polar, the petroleum ether extract was not assessed. Each extract was made into a stock solution at a concentration of 1 mg/mL in methanol. After mixing 2.5 mL of ten-fold diluted F-C reagent with an aliquot of 0.5 mL of the extract solution, the mixture was let to stand for five minutes. The mixture was then supplemented with 2 mL of a 7.5% (w/v) sodium carbonate solution. After vortexing the reaction mixture, it was allowed to sit at room temperature for half an hour in the dark. A UV-Vis spectrophotometer was used to assess the absorbance of the resultant blue-colored complex at a wavelength of 765 nm in comparison to a reagent blank. Gallic acid concentrations ranging from 20 to 100 µg/mL were used to create a standard calibration curve. The total phenolic content was reported as milligrams of gallic acid equivalents per gram of dried extract (mg GAE/g), and all measurements were carried out in triplicate.

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3.8 Total Flavonoid Content

Using quercetin as the reference flavonoid constituent, the aluminum chloride colorimetric test was used to assess the total flavonoid content (TFC). One milligram per milliliter of ethanolic and aqueous extract solutions were made. 0.3 mL of a 5% (w/v) sodium nitrite solution was combined with one milliliter of the extract solution. Five minutes later, 0.3 mL of a 10% (w/v) aluminum chloride solution was added, and the combination was left to react for six minutes. Lastly, 2 mL of 1 M sodium hydroxide solution was added, and distilled water was added to bring the total amount to 10 mL. After a thorough vortex, the mixture was allowed to sit at room temperature for fifteen minutes. At 510 nm, the pinkish-red chromophore's absorbance was measured in comparison to a blank. In the concentration range of 20–100 µg/mL, a standard calibration curve for quercetin was created. The findings were reported as milligrams of quercetin equivalents per gram of dried extract (mg QE/g), and the test was run in triplicate.

3.9 Antioxidant Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test was used to assess the extracts' capacity to scavenge free radicals. A range of working concentrations (20, 40, 60, 80, and 100 µg/mL) were obtained by diluting stock solutions of the ethanolic and aqueous extracts in methanol. A newly made 0.1 mM DPPH solution in methanol was used. One milliliter of each extract dilution and one milliliter of the DPPH solution were combined for the test. After a violent vortex, the mixture was allowed to sit at room temperature for half an hour in the dark. At 517 nm, the absorbance of the resultant solution (A_sample) was measured in comparison to a blank (1 mL methanol + 1 mL extract). An analogous control reaction (A_control) was made by substituting 1 mL of methanol for the extract. Under the same circumstances, ascorbic acid served as the reference standard antioxidant. Using the formula, the radical scavenging activity was determined as the percentage of DPPH inhibition:

$$\text{DPPH Inhibition (\%)} = [(\text{A\_control} - \text{A\_sample}) / \text{A\_control}] \times 100$$

The assay was performed in triplicate for each concentration. The percentage inhibition values were plotted against the extract concentrations, and the IC_{50} value, defined as the concentration of extract required to scavenge 50% of the DPPH radicals, was calculated from the linear regression equation of the dose-response curve. A lower IC_{50} value indicates higher antioxidant potency.

3.10 Antibacterial Activity

Two Gram-positive bacteria, Staphylococcus aureus (MTCC 96) and Bacillus subtilis (MTCC 441), and two Gram-negative bacteria, Escherichia coli (MTCC 443) and Pseudomonas aeruginosa (MTCC 424), were used to test the extracts' in vitro antibacterial activity. The Microbial Type Culture Collection (MTCC) in Chandigarh, India, provided all of the bacterial strains. Before testing, the cultures were kept on nutrient agar slants at 4°C and subcultured in nutrient broth for 24 hours at 37°C.

The first screening of antibacterial activity was done using the agar well diffusion technique. Mueller-Hinton agar plates were prepared, and a sterile cotton swab was used to evenly distribute 100 µL of a standardized bacterial inoculum (0.5 McFarland standard) throughout

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the agar surface. A sterile cork borer was used to punch 6 mm-diameter wells into the seeded agar. To create stock solutions of 50, 100, and 150 mg/mL, the extracts were diluted in 10% dimethyl sulfoxide (DMSO). Each concentration was carefully added to the corresponding wells in a constant amount of 50 µL. After allowing pre-diffusion for half an hour at room temperature, the plates were incubated for twenty-four hours at 37°C. The positive control was ciprofloxacin (5 µg/disc), whereas the negative control was 10% DMSO. Using a ruler, the diameter of the clear zone of inhibition around each well was measured in millimeters after incubation.

In 96-well microtiter plates, the broth microdilution technique was used to calculate the minimum inhibitory concentration (MIC). Each extract was serially diluted twice in Mueller-Hinton broth to obtain a concentration range of 0.5 to 10 mg/mL. Twenty microliters of the bacterial suspension were added to each well. After being sealed, the plates were incubated for 18 to 24 hours at 37°C. Following incubation, each well received 20 µL of 0.015% resazurin dye, and the plates were then incubated for an additional two hours. Bacterial growth was indicated by a shift in color from blue to pink. The minimum inhibitory concentration (MIC) was defined as the extract's lowest concentration that totally stopped apparent bacterial growth by preventing color change.

3.11 Statistical Analysis

To guarantee the accuracy and repeatability of the findings, every experimental test was carried out three times. The results were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to analyze statistical differences between the groups. Tukey's post-hoc multiple comparison test was then used to identify significant differences between means. Statistical significance was defined as a probability value of $p < 0.05$. GraphPad Prism program (Version 9.0, GraphPad program, Inc., San Diego, CA, USA) was used for all statistical calculations and graphical displays.

4. RESULTS

4.1 Extractive Yield

Table 1 shows the % yield, texture, and physical characteristics of the three consecutive Martynia annua leaf extracts. The ethanolic and aqueous extracts produced the most dry residue, whereas the petroleum ether extract produced the least.

Table 1: Physical Characteristics and Percentage Yield of Different Leaf Extracts of Martynia annua L.

Extract	Weight of dried extract (g)	Yield (%, w/w)	Colour	Texture
Petroleum ether	2.16	2.16	Dark green	Waxy semi-solid
Ethanol	14.85	14.85	Dark brown	Sticky solid

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Aqueous	16.42	16.42	Brown	Hygroscopic powder

Initial weight of dried leaf powder: 100 g for each extraction.

4.2 Phytochemical Screening

The ethanolic extract has the greatest variety of secondary metabolites, according to the qualitative analysis. The ethanol extract included high concentrations of alkaloids, flavonoids, phenolic compounds, tannins, and glycosides (+++). While the petroleum ether extract tested positive mainly for steroids and terpenoids, the aqueous extract showed a substantial level of saponins and carbohydrates. Table 2 summarizes the comprehensive semi-quantitative findings.

Table 2: Preliminary Phytochemical Screening of Different Leaf Extracts

Phytoconstituent	Petroleum Ether Extract	Ethanol Extract	Aqueous Extract
Alkaloids	−	++	+
Flavonoids	−	+++	++
Phenolic compounds	−	+++	++
Tannins	−	++	+
Saponins	−	−	+++
Glycosides	−	++	+
Terpenoids	++	+	−
Steroids	++	−	−
Carbohydrates	−	+	+++
Proteins	−	+	++

Notation: − = Absent; + = Weakly present; ++ = Moderately present; +++ = Strongly present.

4.3 Total Phenolic Content

The Folin-Ciocalteu technique was used to calculate the total phenolic content (TPC) of the ethanolic and aqueous extracts. Compared to the aqueous extract, the ethanolic extract had a considerably greater TPC ($p < 0.05$). Because the test is polar, petroleum ether extract was not assessed. Table 3 displays the outcomes.

Table 3: Total Phenolic Content of Different Extracts

Extract	Total Phenolic Content (mg GAE/g dried extract) (Mean ± SD, n=3)
Ethanol	112.45 ± 3.28

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Aqueous	78.32 ± 2.15

GAE: Gallic acid equivalent. Values with different superscript letters are significantly different ($p < 0.05$).

4.4 Total Flavonoid Content

The aluminum chloride colorimetric test was used to measure the total flavonoid content (TFC). The ethanolic extract had a much greater concentration of flavonoids than the aqueous extract, as was shown for TPC (Table 4).

Table 4: Total Flavonoid Content of Different Extracts

Extract	Total Flavonoid Content (mg QE/g dried extract) (Mean ± SD, n=3)
Ethanol	**68.74 ± 2.01**
Aqueous	**41.56 ± 1.87**

QE: Quercetin equivalent. The difference between the two extracts was statistically significant ($p < 0.05$).

4.5 Antioxidant Activity

For both the ethanolic and aqueous extracts, the DPPH radical-scavenging test demonstrated a distinct dose-dependent rise in the percentage of inhibition across the concentration range of 20–100 µg/mL. At every dose examined, the ethanolic extract demonstrated an exceptional ability to scavenge radicals. The linear regression of the dose-response curves was used to get the IC_{20} values, which indicate the concentration needed to block 50% of DPPH radicals. Ascorbic acid, a common antioxidant, had the strongest efficacy. Table 5 displays the IC_{50} values and % inhibition.

Table 5: DPPH Radical-Scavenging Activity of Different Extracts

Concentration (µg/mL)	Ethanol Extract (% Inhibition, Mean ± SD)	Aqueous Extract (% Inhibition, Mean ± SD)	Ascorbic Acid (% Inhibition, Mean ± SD)
20	28.54 ± 1.18	15.34 ± 0.92	45.82 ± 1.45
40	47.21 ± 1.76	26.12 ± 1.05	67.23 ± 1.98
60	63.85 ± 2.31	38.72 ± 1.42	82.54 ± 2.21
80	78.42 ± 2.65	52.48 ± 1.86	92.11 ± 1.76
100	88.95 ± 2.93	67.41 ± 2.14	96.33 ± 1.58
IC_{50} (µg/mL)	45.23	74.85	25.62

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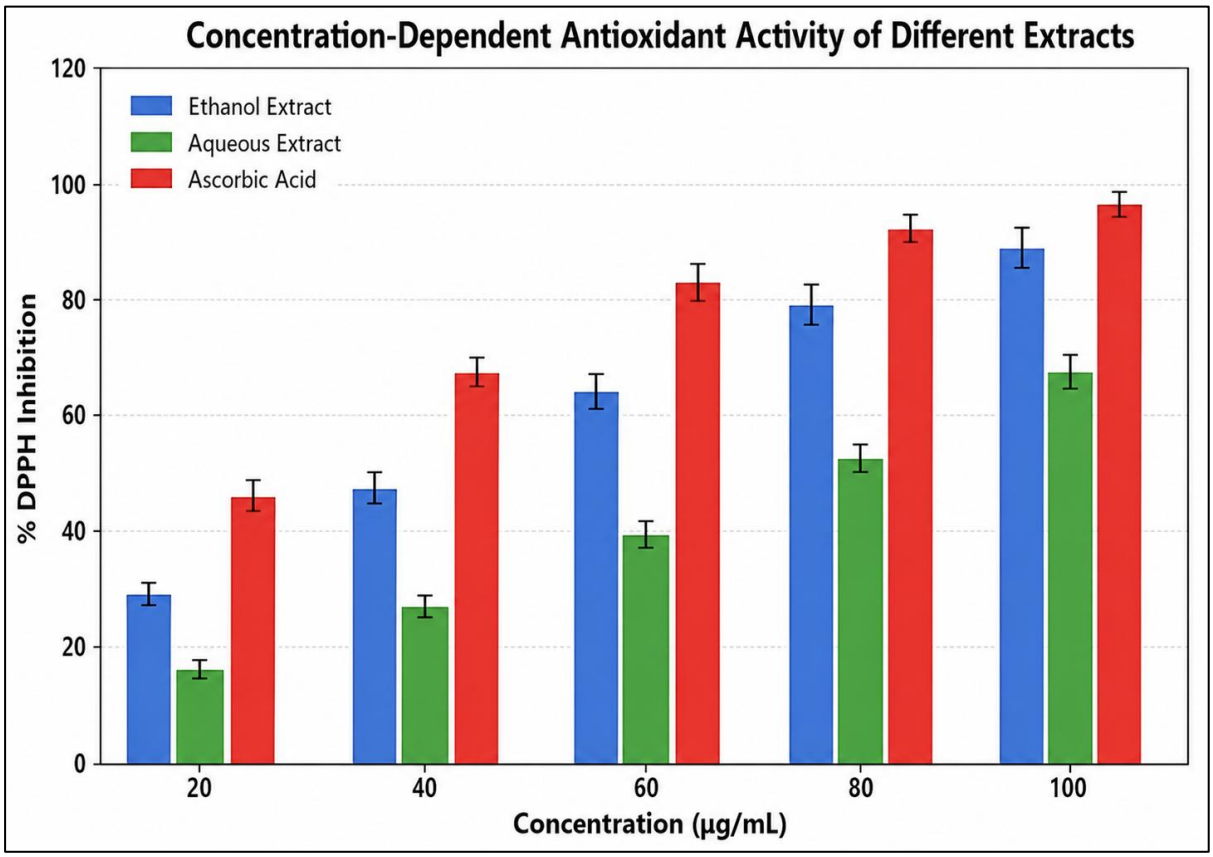

Figure 2: Concentration-Dependent Antioxidant Activity of Different Extracts. A bar chart comparing the percentage DPPH inhibition at 20, 40, 60, 80, and 100 µg/mL for ethanolic extract, aqueous extract, and ascorbic acid standard.

4.6 Antibacterial Activity

The ethanolic extract showed the widest and strongest antibacterial activity against all tested pathogens, according to the agar well diffusion experiment. Compared to the Gram-negative species, the Gram-positive bacteria—especially Bacillus subtilis—were more vulnerable. There was no discernible zone of inhibition produced by the petroleum ether extract. Table 6 lists the zones of inhibition for the maximum measured concentration (150 mg/mL).

Table 6: Antibacterial Activity of Different Leaf Extracts (Zone of Inhibition in mm at 150 mg/mL extract concentration)

Bacterial Strain	Ethanol Extract	Aqueous Extract	Ciprofloxacin (5 µg/disc)	Negative Control (10% DMSO)
Staphylococcus aureus	16.23 ± 0.47	12.38 ± 0.55	24.31 ± 0.82	0.00
Bacillus subtilis	18.14 ± 0.68	14.21 ± 0.52	26.12 ± 0.71	0.00

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Escherichia coli	13.52 ± 0.41	10.15 ± 0.31	30.21 ± 0.95	0.00
Pseudomonas aeruginosa	11.85 ± 0.59	9.23 ± 0.42	28.47 ± 0.78	0.00

Values are mean zone diameter ± SD (n=3), including the 6 mm well diameter. Petroleum ether extract showed no inhibition (0 mm).

The ethanolic extract's enhanced efficacy was further validated by the minimum inhibitory concentration (MIC) values, which showed the lowest MIC (0.625 mg/mL) against B. subtilis. P. aeruginosa was the most resistant bacterium tested, and the aqueous extract had higher MIC values (Table 7).

Table 7: Minimum Inhibitory Concentration (MIC) of Active Extracts

Bacterial Strain	MIC – Ethanol Extract (mg/mL)	MIC – Aqueous Extract (mg/mL)
Staphylococcus aureus	1.25	2.50
Bacillus subtilis	0.625	1.25
Escherichia coli	2.50	5.00
Pseudomonas aeruginosa	5.00	>10.00

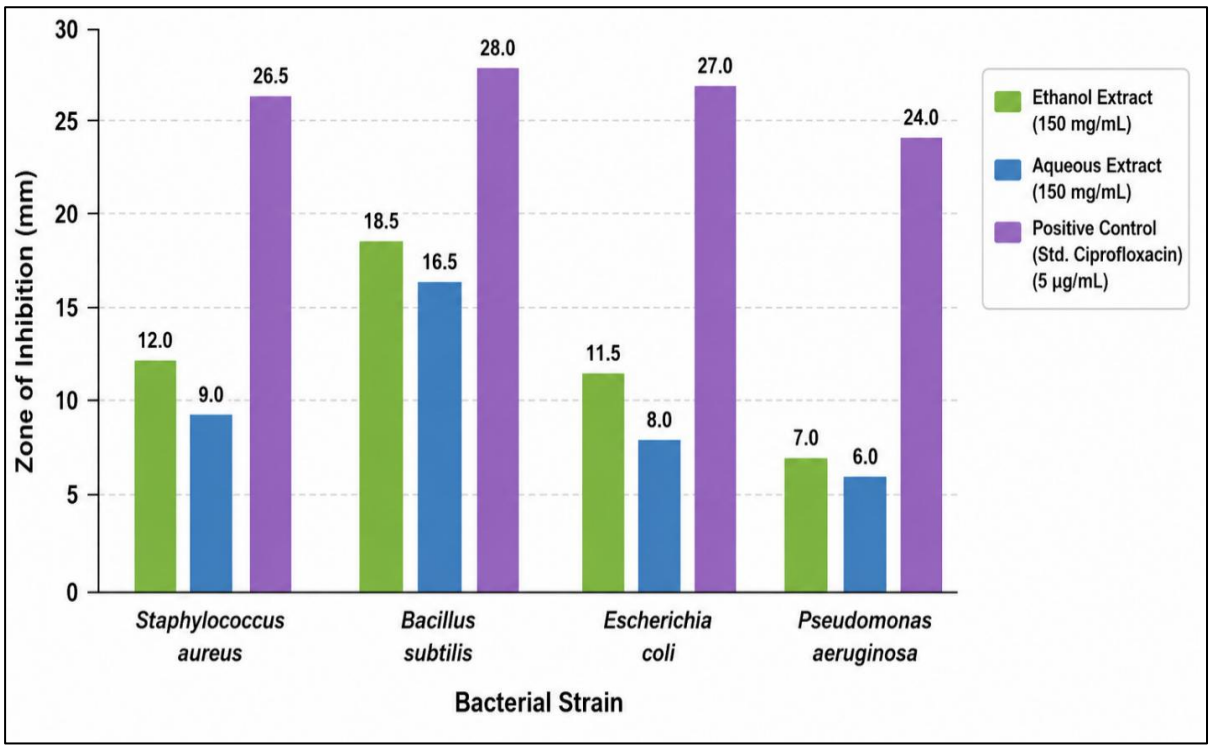

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Figure 3: Comparison of Antibacterial Activity of Different Extracts. A clustered bar chart depicting the zone of inhibition (mm) produced by the ethanolic and aqueous extracts at 150 mg/mL against the four bacterial strains, alongside the positive control.

5. DISCUSSION

5.1 Main Findings

The phytochemical profile and biological activity of Martynia annua L. leaf extracts made with different polarity solvents were methodically assessed in this study. The preparation that performed the best out of the three extracts was the ethanolic extract. In addition to having the greatest total phenolic content (112.45 mg GAE/g) and total flavonoid content (68.74 mg QE/g), it also showed the best antioxidant potential, as seen by the DPPH assay's lowest IC•₀ value of 45.23 µg/mL. At the same time, the ethanol extract showed the most antibacterial activity against all tested pathogens, producing the biggest zones of inhibition and the lowest MIC values. In the polar tests, the petroleum ether extract was essentially inert whereas the aqueous extract had modest bioactivities, confirming the crucial importance of extraction solvent in assessing a medicinal plant's pharmacological potential.

5.2 Extraction Yield

The polarity of the solvent and the characteristics of the soluble phytoconstituents are directly responsible for the notable variations in extractive yields. Since water is a highly polar solvent, it extracted the greatest amount of material (16.42%), which included polar molecules that are common in leaf tissues, such as proteins, carbohydrates, saponins, and certain glycosides. With an intermediate polarity of 14.85%, ethanol effectively extracted a wide range of bioactive compounds, including flavonoids and phenolics. Due to its preferential dissolution of non-polar lipids, waxes, steroids, and chlorophyll, the petroleum ether extract exhibited the lowest yield (2.16%). This yield pattern is consistent with the general idea that a solvent's effectiveness is determined by how well it dissolves the main structural and storage elements of the plant matrix.

5.3 Phytochemical Constituents

Ethanol is the best solvent for extracting pharmacologically significant phytochemicals, according to the qualitative screening, which supported the quantitative findings. Flavonoids, phenolic chemicals, tannins, alkaloids, and glycosides were all significantly detected in the ethanolic extract. The medicinal properties of several groups of secondary metabolites are well acknowledged. Because of their redox characteristics, which enable them to function as reducing agents, hydrogen donors, and singlet oxygen quenchers, phenolic compounds and flavonoids are classic antioxidants. The antibacterial and wound-healing activities of tannins are supported by their astringent and protein-binding capacities. Alkaloids are a broad class of compounds with significant physiological effects, such as antibacterial action. The petroleum ether extract's lack of antioxidant and antibacterial activity in the polar tests is explained by the presence of just steroids and terpenoids, while the aqueous extract's substantial content of saponins and carbohydrates explains its moderate bioactivity.

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5.4 Antioxidant Activity

For both polar extracts, the DPPH test findings showed a definite, dose-dependent radical-scavenging action, with activity rising in direct proportion to concentration. Although it was still greater than that of the reference standard ascorbic acid (25.62 µg/mL), the IC•γ value, a crucial indicator of antioxidant effectiveness, was much lower for the ethanolic extract (45.23 µg/mL) than for the aqueous extract (74.85 µg/mL). The much increased levels of phenolic and flavonoid chemicals found in the ethanol extract are directly related to this. Free radicals are neutralized by the hydroxyl groups on phenolic and flavonoid nuclei, according to the recognized structure-activity connection. Consequently, an extract with a higher concentration of these substances, such as the ethanolic extract, would naturally have more potent antioxidant action. This relationship confirms that the main components of M. annua leaves' antioxidant ability are phenolics and flavonoids.

5.5 Antibacterial Activity

The antibacterial tests demonstrated a clear selectivity in the extracts' actions. For every bacterial strain, the ethanolic extract consistently exhibited greater zones of inhibition and lower MIC values than the aqueous extract. Gram-negative Pseudomonas aeruginosa was the most resistant bacteria, whereas Gram-positive Bacillus subtilis was the most susceptible. The structural variations in the bacterial cell wall are essentially responsible for this variable susceptibility. Bioactive chemicals may more easily penetrate the thick, porous peptidoglycan coating of gram-positive bacteria. Gram-negative bacteria, on the other hand, have an outer lipopolysaccharide membrane that functions as a strong permeability barrier, keeping out a lot of big, hydrophobic compounds. Additionally, it was evident that the extract's action was concentration-dependent, with larger extract loading leading to stronger inhibition. The extract's multi-component structure provides a viable template for combination therapy, despite its lower potency compared to the broad-spectrum antibiotic ciprofloxacin. Flavonoids, tannins, and alkaloids work together to impair membrane integrity, inhibit enzymes, and interfere with genetic material, which is probably how the observed activity is mediated.

5.6 Possible Biological Mechanism

The many phytochemical pools found in the extracts interact synergistically to produce the reported antioxidant and antibacterial properties. Phenolics and flavonoids are thought to have antibacterial properties through a number of mechanisms, including deactivation of important metabolic enzymes, chelation of metal ions necessary for microbial growth, disruption of the cytoplasmic membrane that allows cellular contents to leak out, and inhibition of nucleic acid synthesis. Tannins work by coagulating the cell wall and attaching to microbial surface proteins and adhesins to inhibit adhesion and biofilm formation. Alkaloids have the ability to inhibit ion channels or intercalate with bacterial DNA. Compared to single-molecule antibiotics, this multi-target approach reduces the likelihood of bacterial resistance developing. It is important to note that while these in vitro findings are encouraging, further in vivo validation is necessary since they do not immediately transfer into a therapeutic treatment for infectious illnesses.

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5.7 Strength of the Study

This study's methodological approach has a number of noteworthy advantages. First, a thorough phytochemical and bioactivity fingerprint of the leaf is obtained by comparing the use of many increasingly polar solvents; ethanol is shown to be the best extraction solvent. Second, by offering quantitative estimations of total phenolic and flavonoid contents, the research goes beyond qualitative screening and enables a significant dose-activity connection. Third, a dose-response design was used in the antioxidant assessment to provide exact IC_{20} values, allowing for a thorough comparison. Fourth, a disc-diffusion screening approach and a quantitative broth microdilution MIC determination against clinically relevant Gram-positive and Gram-negative microorganisms were used in the two-tiered antibacterial testing. Lastly, all experiments were conducted in triplicate and the results were robustly analyzed using the proper inferential statistics.

5.8 Limitations

Notwithstanding these advantages, the study has many drawbacks. Although crude multi-component extracts are useful for researching synergism, they make it impossible to pinpoint the precise bioactive molecule causing the action. Despite encompassing common pathogenic groups, the panel of bacterial strains has a narrow focus and excludes drug-resistant variations that have been clinically identified. Advanced analytical characterisation that would have detected certain marker molecules, such HPLC, GC-MS, or LC-MS fingerprinting, was absent from the research. The intricate pharmacokinetic elements of absorption, distribution, metabolism, and excretion that control in vivo effectiveness were also not taken into consideration since all biological activities were evaluated only in vitro. Lastly, no toxicity assessment—either in vitro cytotoxicity or in vivo acute toxicity—was carried out, which is a crucial need for determining the extracts' safety profile for any potential therapeutic use.

6. CONCLUSION

This study's main goal was to assess the phytochemical components, antibacterial capability, and antioxidant activity of various solvent extracts made from the aerial portions of Martynia annua L. leaves. The study showed that the yield, phytochemical profile, and biological effectiveness of the extract are all significantly impacted by the extraction solvent selection. The ethanolic extract continuously showed the most encouraging outcomes out of the petroleum ether, ethanolic, and aqueous extracts. The ethanol extract included a wide variety of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, and glycosides, according to phytochemical screening. Quantitative tests confirmed this qualitative richness, with the ethanol extract exhibiting the greatest total phenolic and total flavonoid contents. Accordingly, in the DPPH radical-scavenging experiment, the ethanol extract showed the best in vitro antioxidant activity with the lowest $IC_{\bullet 0}$ value, suggesting a substantial dose-dependent free-radical quenching capability. Bacillus subtilis was the most vulnerable organism to the ethanolic extract's high antibacterial activity, which inhibited both Gram-positive and Gram-negative harmful bacteria. In the polar experiments, the aqueous extract demonstrated modest activity whereas the petroleum ether extract was essentially inert. These

results establish the ethanolic leaf extract as a good source of antioxidant and antibacterial chemicals and provide a scientific foundation for the traditional medical usage of Martynia annua. The study emphasizes how crucial solvent selection is for phytopharmacological studies. To fully realize the therapeutic potential of this plant, however, more research is necessary, including bioassay-guided isolation and characterization of the particular bioactive molecules using cutting-edge methods like HPLC and GC-MS, clarification of their precise mechanisms of action, and assessment of in vivo efficacy and toxicity profiles.

7. FUTURE SCOPE

A fundamental knowledge of the phytochemical profile and biological activity of Martynia annua L. leaf extracts has been developed by the current study. Future studies, however, still have a number of options for advancing these discoveries in the direction of possible therapeutic use.

To pinpoint the precise bioactive compounds causing the reported activities, advanced analytical characterisation is necessary. Key individual phenolic and flavonoid marker chemicals may be fingerprinted and quantified using high-performance liquid chromatography (HPLC). While liquid chromatography-mass spectrometry (LC-MS) can offer a thorough metabolomic profile by identifying a larger range of semi-polar and polar compounds with high sensitivity and structural information, gas chromatography-mass spectrometry (GC-MS) is advised for the identification of volatile and non-polar constituents present in the active extracts.

Bioassay-guided fractionation and separation of the most active chemicals should be carried out after analytical profiling. The individual and combined biological impacts of the separated molecules may then be thoroughly examined. The safety margin of the extracts and isolated compounds must be established by further toxicity assessment, which includes both in vivo acute and sub-acute oral toxicity tests in animal models and in vitro cytotoxicity on normal cell lines.

Given that biofilm-associated illnesses are infamously difficult to treat, extending the biological assessment to include anti-biofilm effectiveness against pathogenic bacteria is a therapeutically important addition. Additionally, in vivo research using suitable animal models of oxidative stress and bacterial infection to evaluate pharmacological activity, bioavailability, and pharmacokinetics is necessary to corroborate the encouraging in vitro antioxidant and antibacterial findings. Lastly, the creation of topical or oral herbal formulations, such as creams, ointments, or capsules containing standardized M. annua extracts, might be investigated for commercial and clinical translation after satisfactory toxicological and in vivo validation.

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