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Phytochemical, Antioxidant and Antibacterial Evaluation of Aerial-Part Extracts of Tanacetum parthenium L.

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ABSTRACT
Background: Tanacetum parthenium L., often known as feverfew, is a medicinal plant that has long been used to prevent migraines and treat inflammatory diseases. Nevertheless, there is still a lack of comparative information about the phytochemical and biological characteristics of its aerial-part extracts. The purpose of this research was to assess the phytochemical components, antibacterial potential, and antioxidant activity of petroleum ether, ethanol, and aqueous extracts of T. parthenium aerial parts. Methods: The extracts were submitted to qualitative phytochemical screening after successive solvent extraction. Folin-Ciocalteu and aluminum chloride techniques were used to measure the total phenolic content (TPC) and total flavonoid content (TFC), respectively. The DPPH radical-scavenging test was used to measure antioxidant activity, while broth microdilution and agar well diffusion methods were used to measure antibacterial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa. Findings: The ethanol extract had the strongest DPPH scavenging activity (IC_{50} 52.31 ± 1.49 µg/mL) and the greatest TPC (148.72 ± 3.15 mg GAE/g) and TFC (62.34 ± 1.92 mg QE/g). Additionally, it showed the biggest zones of inhibition, and the most vulnerable organism was S. aureus (19.4 ± 0.8 mm; MIC 1.56 mg/mL). While the petroleum ether extract was essentially inert, the aqueous extract exhibited modest activity. In conclusion, the ethanol extract of the aerial portions of T. parthenium is a rich source of phenolic compounds with notable antibacterial and antioxidant properties in vitro, indicating the need for more research to isolate bioactive components and assess safety.

Keywords: Tanacetum parthenium, aerial-part extract, phytochemical screening, antioxidant activity, antibacterial activity

1. INTRODUCTION

Since ancient times, medicinal plants have been an essential part of healthcare systems all throughout the globe. According to estimates from the World Health Organization, 80% of people in underdeveloped nations still get their main medical treatment from traditional plant-based remedies [1,2]. The belief that natural products are safer, more economical, and often successful against infectious and chronic illnesses when conventional medicines fail is the

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driving force for the resurgence of interest in herbal therapy worldwide [3]. This dependence highlights the need to turn ethnobotanical knowledge into evidence-based phytopharmaceuticals by rigorously verifying the therapeutic claims linked to medicinal flora [4].

Secondary metabolites—bioactive substances that are not directly engaged in growth or reproduction but function as defense molecules against herbivores, pathogens, and environmental stress—are largely responsible for the medicinal potential of medicinal plants [5]. Phenolics, flavonoids, alkaloids, terpenoids, and tannins are among the phytochemicals that display an impressive range of biological activities, including antioxidant, antibacterial, anti-inflammatory, and anticancer properties [6,7]. These metabolites provide a rich reservoir for drug development due to their structural diversity and synergistic interactions. As a result, a crucial stage in the creation of standardized herbal medicines is the qualitative and quantitative assessment of secondary metabolites in plant extracts [8,9].

Feverfew, or Tanacetum parthenium L. (syn. Chrysanthemum parthenium), is a perennial plant in the Asteraceae family [10]. Although it originated in the Balkan Peninsula, the plant has spread across Europe, North America, and certain regions of Asia [11]. It grows to a height of 30 to 60 cm and has daisy-like composite flower heads with yellow disc florets and white ray florets, as well as very fragrant, pinnatisect leaves [12]. The traditional source of drugs has been the aerial portions, which include leaves, stems, and inflorescences. In European folk medicine, feverfew has traditionally been used to treat fevers, rheumatoid arthritis, irregular menstruation, and migraine prevention [13,14]. Its effectiveness in lowering the frequency and intensity of migraine episodes has been validated by contemporary clinical research; this effect is mostly attributed to the sesquiterpene lactone parthenolide [15,16]. In many preclinical models, preparations of the aerial portions have shown anti-inflammatory, antibacterial, and cytotoxic qualities in addition to their antimigraine efficacy [17,18]. Standardized extract extraction is encouraged by the European Medicines Agency and the British Herbal Pharmacopoeia, which recognize the aerial sections as the official part utilized [19,20].

The antioxidant and antibacterial properties of several solvent extracts of T. parthenium aerial parts are still poorly understood, despite the well-established anti-inflammatory and antimigraine uses [21]. Although there are few systematic comparison studies utilizing solvents of different polarity, it is believed that the polyphenolic fraction plays a major role in scavenging free radicals [22,23]. Furthermore, feverfew's high terpenoid and phenolic profile makes it a good option for investigating plant-derived antimicrobials in light of the growing threat of antibiotic resistance [24,25]. Correlating the chemical composition with the reported bioactivities requires a thorough phytochemical screening in addition to quantifying the overall phenolic and flavonoid contents [26]. In addition to confirming the conventional claims, this assessment will assist in determining the most effective extract for possible development as a natural antioxidant or antibacterial agent.

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Given the aforementioned, the current research was created with the following goals in mind:

Aim

To evaluate the phytochemical constituents, antioxidant activity and antibacterial potential of different solvent extracts of the aerial parts of Tanacetum parthenium L.

2. OBJECTIVES

1. To prepare different solvent extracts from the aerial parts of T. parthenium.
2. To determine the extractive yield of each solvent extract.
3. To conduct qualitative phytochemical screening of the extracts.
4. To determine the total phenolic content of the extracts.
5. To determine the total flavonoid content of the extracts.
6. To evaluate the antioxidant activity of the extracts using appropriate in vitro assays.
7. To evaluate the antibacterial activity of the extracts against selected pathogenic bacterial strains.
8. To compare the phytochemical profiles and bioactivities of the different solvent extracts.

3. MATERIALS AND METHODS

3.1 Study Design

The current research was created as an experimental laboratory investigation conducted at the Department of Pharmaceutical Sciences, Faculty of Pharmacy, RKDF University, Bhopal, Madhya Pradesh, India. Every experiment was carried out at the same institution's Microbiology Laboratory and Pharmacognosy Research Laboratory. The research was carried out from March 2024 to August 2024, a span of six months.

3.2 Collection of Plant Material

In March 2024, fresh aerial portions of Tanacetum parthenium L. were gathered at the The plant material was collected from the Medicinal Plant Garden located on the RKDF University Campus, Bhopal, Madhya Pradesh, India (approximately 23°15′ N latitude and 77°25′ E longitude; elevation about 500–520 m above mean sea level). The plant material included soft stems, fully opened blooms, and healthy, disease-free leaves. In order to maximize the accumulation of secondary metabolites, only plants that were developing rapidly during the blooming stage were chosen. The gathered sample was promptly put in sterile polythene bags, brought to the lab, and processed that same day.

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3.3 Plant Authentication

Figure 3.1. Morphological appearance of Tanacetum parthenium (L.) Sch.Bip. showing leaves, stems, and inflorescenc

Figure 3.1 shows the morphological appearance of the Tanacetum parthenium aerial pieces that were collected. Professor Deepak Vyas, a taxonomist in the Department of Botany at Dr. Harisingh Gour University, Sagar confirmed the plants identify. After preparing a herbarium specimen, the authenticity certificate (Certificate No. HU/BOT/2024/TP-042) was acquired. For future reference, the voucher specimen (Voucher No. HU-PHCOG-012/24) was placed in the institutional herbarium.

3.4 Preparation of Plant Material

The new aerial parts were rinsed with distilled water after being properly cleaned under running tap water to get rid of any dust, dirt, and other debris. After washing, the material was spread out on blotting paper and shade-dried at room temperature (25 ± 2 °C) with sufficient airflow until it was crisp. Any discolored, insect-damaged, or senescent pieces were physically sorted and disposed of during the drying process. To achieve a consistent particle size, the fully dry material was coarsely ground using a mechanical grinder (Model MX AC400, Panasonic) and then run through a 40 mesh sieve (BSS). Before being extracted, the sieved powder was placed in an airtight, amber-colored glass container and kept out of direct sunlight in a cold, dry location.

3.5 Preparation of Extracts

Three increasingly polar solvents were used in consecutive extractions: distilled water, ethanol (95% v/v), and petroleum ether (60–80 °C). 500 g of precisely measured dry powder was first defatted by macerating it in a conical flask with a stopper using 1.5 L of petroleum ether. For 72 hours, the mixture was shaken at room temperature on an orbital shaker set at 120 rpm with sporadic hand stirring. Whatman No. 1 filter paper was used to filter the content after

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maceration. Under the same circumstances, the marc was air dried and then extracted using 1.5 L of ethanol. Finally, 1.5 L of distilled water was used to extract the marc that remained after ethanol extraction, once more for 72 hours at room temperature. A rotary vacuum evaporator (Rotavapor R 300, Buchi, Switzerland) was used to concentrate each filtrate under low pressure at a temperature of no more than 40 °C. In order to achieve a consistent weight, the concentrated extracts were further dried at 37 °C in a hot air oven. For further examination, the dried extracts were kept at 4 °C in sterile, labeled glass bottles.

The extractive yield (percentage, w/w) was calculated using the following formula [9]:

$$\text{Extractive Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant powder}} \times 100$$

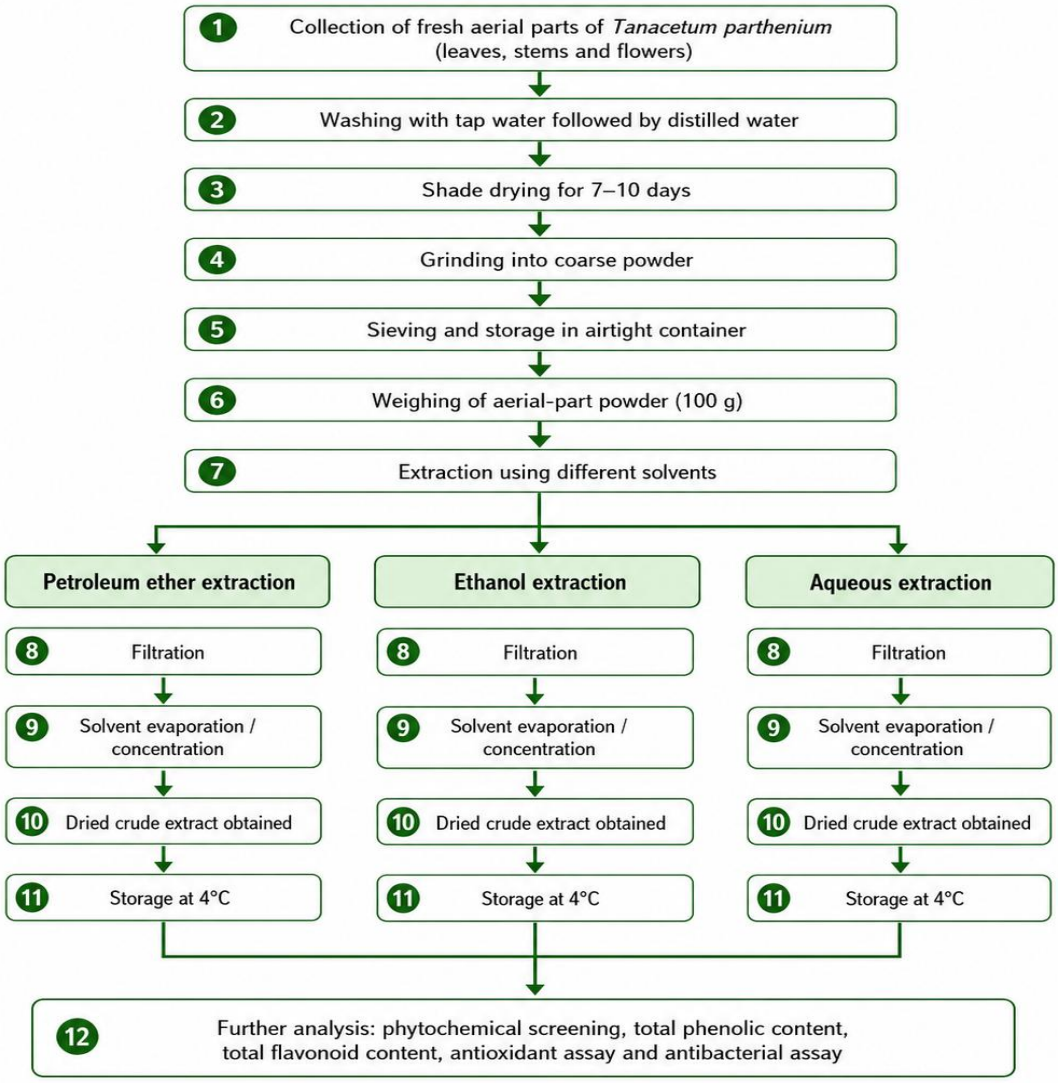

Figure 3.2. Schematic flowchart of the extraction process for the aerial parts of Tanacetum parthenium.

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3.6 Preliminary Phytochemical Screening

To find the existence of significant secondary metabolites, a qualitative phytochemical screening of all three extracts was carried out using conventional colorimetric and precipitation procedures. The tests were conducted in compliance with recognized guidelines. Dragendorff's and Mayer's tests were used to identify alkaloids; Shinoda and alkaline reagent tests for flavonoids; ferric chloride tests for phenolics; lead acetate and gelatin tests for tannins; frothing tests for saponins; Keller-Kiliani tests for cardiac glycosides; Salkowski's test for terpenoids; Liebermann-Burchard tests for steroids; Molisch's and Fehling's tests for carbohydrates; and biuret and ninhydrin tests for proteins. Based on the strength of the distinctive color or precipitate seen, the results were classified as nonexistent (−), faint (+), moderate (++) or strong (+++).

3.7 Total Phenolic Content

With a few modest adjustments, the Folin–Ciocalteu (F C) technique as outlined by Singleton et al. [26] was used spectrophotometrically to measure total phenolic content (TPC). To put it briefly, 1.5 mL of 10-fold diluted F C reagent was combined with 200 µL of suitably diluted extract (or standard gallic acid solution). 1.5 mL of a 7.5% (w/v) sodium carbonate solution was added after a 5-minute incubation period at room temperature. After vortexing the mixture, it was left to stand at 40 °C for 30 minutes in the dark. A UV Vis spectrophotometer (Model UV 1900i, Shimadzu, Japan) was used to detect absorbance at 765 nm in comparison to a reagent blank. Gallic acid (1–100 µg/mL; R2 = 0.9992) was used to create a calibration curve. Gallic acid equivalents per gram of dried extract (mg GAE/g) were used to express TPC. Every calculation was carried out in triplicate.

3.8 Total Flavonoid Content

The aluminum chloride colorimetric technique was used to quantify the total flavonoid content (TFC) [30]. 1.5 mL of methanol, 100 µL of 10% (w/v) aluminum chloride, 100 µL of 1 M potassium acetate, and 2.8 mL of distilled water were combined with an aliquot of 500 µL of extract (or standard quercetin solution). After 30 minutes of room temperature incubation, the mixture's absorbance at 415 nm was measured. Quercetin (5–200 µg/mL; R2 = 0.9987) was used to create a standard curve. TFC was measured as mg QE/g, or milligrams of quercetin equivalents per gram of dried extract. Every measurement was done three times.

3.9 Antioxidant Activity

Using the 1,1 diphenyl 2 picrylhydrazyl (DPPH) radical scavenging experiment, antioxidant activity was assessed using a slightly modified version of Brand Williams et al.'s approach [31]. Each extract's stock solutions (1 mg/mL) and the reference standard ascorbic acid were made in methanol. One milliliter of each serial dilution (20–200 µg/mL) was combined with two milliliters of newly manufactured 0.1 mM DPPH methanolic solution. After completely vortexing the reaction mixture, it was allowed to sit at room temperature for half an hour in the dark. The resultant solution's absorbance was measured at 517 nm in comparison to a methanol

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blank. Simultaneously, a control with a DPPH solution devoid of extract was conducted. The formula was used to determine the percentage of DPPH radical inhibition:

$$\text{DPPH Inhibition (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where A_sample is the absorbance of the DPPH solution with extract or standard, and A_control is the absorbance of the DPPH solution without extract. Plotting percentage inhibition versus extract concentration allowed for the determination of the concentration needed to scavenge 50% of DPPH radicals (IC_{50}). Every experiment was carried out in triplicate, with ascorbic acid serving as a positive control.

3.10 Antibacterial Activity

Two Gram positive bacteria (Staphylococcus aureus MTCC 96, Bacillus subtilis MTCC 441) and two Gram negative bacteria (Escherichia coli MTCC 443, Pseudomonas aeruginosa MTCC 424) were used to test the antibacterial activity of the three extracts. The Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technology in Chandigarh, India, provided all of the test organisms. The cultures were subcultured once a month and kept at 4 °C on nutrient agar slants.

For the first screening, the agar well diffusion technique was used [32]. In short, a sterile cotton swab was used to evenly distribute 100 µL of each standardized bacterial inoculum (0.5 McFarland standard; about 1.5×10^2 CFU/mL) over Mueller Hinton agar plates. A sterile cork borer was used to punch 6 mm diameter wells into the agar, and 50 µL of each extract (pre-dissolved in 10% dimethyl sulfoxide, DMSO, to produce a concentration of 100 mg/mL) was distributed into the wells. After allowing diffusion for 30 minutes at room temperature, the plates were incubated for 18–24 hours at 37 °C. The positive control was ciprofloxacin (30 µg/disc), whereas the negative control was 10% DMSO. Following incubation, a clear ruler was used to measure the sizes of the zones of inhibition, including the well diameter, to the closest millimeter.

According to Eloff [28], the broth microdilution technique in 96-well microtitre plates was used to assess the minimum inhibitory concentration (MIC) for extracts that showed significant activity. Ten microliters of the bacterial suspension were added to each well after two-fold serial dilutions of each extract (100 to 0.78 mg/mL) were made in Mueller Hinton broth. To find bacterial growth, resazurin dye (0.015%) was applied to the plates after they were incubated at 37 °C for 24 hours. The lowest concentration with no discernible color change was noted as the MIC. Every antimicrobial test was run in triplicate.

3.11 Statistical Analysis

Every experiment was carried out in triplicate, and the mean ± standard deviation (SD) was used to describe the findings. One-way analysis of variance (ANOVA) and Tukey's post hoc test for multiple comparisons were used to analyze the data. Statistical significance was defined

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as a probability level of *p* < 0.05. For statistical calculation and graphical depiction, GraphPad Prism version 9.5.0 (GraphPad Software, San Diego, CA, USA) was used.

4. RESULTS

4.1 Extractive Yield

Three extracts with different physical properties and percentage yields were obtained by successively extracting the aerial portions of Tanacetum parthenium L. using petroleum ether, ethanol, and distilled water. The aqueous extract was a yellowish brown, hygroscopic powder, the petroleum ether extract was a dark green, semi-solid waxy mass, and the ethanol extract was a dark brown, viscous sticky residue. Table 1 summarizes the dry extract weights and associated percentage yields. The petroleum ether extract produced the lowest yield (4.91 ± 0.24%), while the aqueous extract produced the greatest yield (22.14 ± 0.87%), followed by the ethanol extract (15.38 ± 0.62%). The yields of the three extracts differed statistically significantly, according to a one-way ANOVA ($F = 142.3$, *p* < 0.001).

Table 1: Physical Characteristics and Extractive Yield of Different Aerial-Part Extracts of Tanacetum parthenium L.

Extract	Colour	Consistency	Dry Extract Weight (g/500 g powder)	Yield (% w/w)†
Petroleum ether	Dark green	Semi-solid waxy	24.55 ± 1.18	4.91 ± 0.24[a]
Ethanol	Dark brown	Viscous sticky	76.90 ± 3.10	15.38 ± 0.62[b]
Distilled water	Yellowish-brown	Hygroscopic powder	110.70 ± 4.35	22.14 ± 0.87[c]

† Values expressed as mean ± SD of triplicate determinations. Means with different superscript letters within a column differ significantly (*p* < 0.05, Tukey's post-hoc test).

4.2 Phytochemical Screening

The distribution of secondary metabolites varied significantly amongst the three extracts, according to qualitative phytochemical screening. Phenolics, flavonoids, terpenoids, and alkaloids were all strongly present in the ethanol extract, which tested positive for the widest range of phytoconstituents. Terpenoids and steroids were the main components of the petroleum ether extract, while saponins, glycosides, proteins, and carbohydrates were abundant in the aqueous extract. Both the ethanol and aqueous extracts had substantial levels of tannins, whereas the petroleum ether extract contained none. Alkaloids and cardiac glycosides were missing in petroleum ether and just slightly positive in the aqueous extract. Table 2 presents the findings in a semi-quantitative manner.

Table 2: Preliminary Phytochemical Profile of Different Extracts

Phytochemical	Petroleum ether	Ethanol	Distilled water
Alkaloids	−	++	+
Flavonoids	−	+++	+

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Phenolics	−	+++	++
Tannins	−	++	++
Saponins	−	+	+++
Cardiac glycosides	−	++	+
Terpenoids	+++	++	−
Steroids	++	+	−
Carbohydrates	−	+	+++
Proteins	−	−	++

Intensity: (+++) strong; (++) moderate; (+) weak; (−) absent.

4.3 Total Phenolic Content

The three extracts had considerably different total phenolic content (TPC) (*p*<0.001). The maximum phenolic content was found in the ethanol extract (148.72 ± 3.15 mg GAE/g extract), which was followed by the aqueous extract (87.45 ± 2.41 mg GAE/g extract). Only minor levels of phenolics (11.63 ± 0.89 mg GAE/g extract) were present in the petroleum ether extract. Over the range of 1–100 µg/mL, the gallic acid standard curve was linear (R2 = 0.9992). Table 3 summarizes these results.

Table 3: Total Phenolic Content of Different Extracts

Extract	TPC (mg GAE/g dry extract)†
Petroleum ether	11.63 ± 0.89^{a}
Ethanol	148.72 ± 3.15^{b}
Distilled water	87.45 ± 2.41^{c}

† Mean ± SD (n = 3). Different superscript letters indicate significant differences (*p* < 0.05).

4.4 Total Flavonoid Content

TPC and total flavonoid content (TFC) showed comparable trends. The maximum concentration of flavonoids was found in the ethanol extract (62.34 ± 1.92 mg QE/g extract), which was significantly higher than the aqueous extract (28.17 ± 1.05 mg QE/g extract). The petroleum ether extract contained hardly little flavonoids (1.04 ± 0.11 mg QE/g extract) (Table 4). The calibration curve for quercetin was linear, with R2 = 0.9987.

Table 4: Total Flavonoid Content of Different Extracts

Extract	**TFC (mg QE/g dry extract)†**
Petroleum ether	1.04 ± 0.11^{a}
Ethanol	62.34 ± 1.92^{b}
Distilled water	28.17 ± 1.05^{c}

† Mean ± SD (n = 3). Different superscripts indicate significant differences (*p* < 0.05).

4.5 Antioxidant Activity

The DPPH radical was scavenged by all three extracts in a concentration-dependent manner (Figure 1). Across all tested concentrations (20–200 µg/mL), the ethanol extract consistently showed the highest percentage inhibition, almost matching the activity of the reference

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standard, ascorbic acid. The ethanol extract showed 89.76 ± 2.13% inhibition at the maximum concentration (200 µg/mL), whereas ascorbic acid showed 96.18 ± 0.94%. While the petroleum ether extract had limited action (38.15 ± 1.56% at the same concentration), the aqueous extract demonstrated considerable scavenging (65.42 ± 1.87% at 200 µg/mL).

Table 5 displays the IC • values that were computed using the dose response curves. The aqueous extract (128.64 ± 3.27 µg/mL), petroleum ether extract (298.45 ± 5.83 µg/mL), and ethanol extract (52.31 ± 1.49 µg/mL) showed the greatest antioxidant capacity. Ascorbic acid's IC^{2}_{2} was 18.22 ± 0.76 µg/mL. All extracts' IC•₀ values were found to vary considerably from the standard and from each other, according to statistical analysis (*p*<0.001).

Table 5: DPPH Radical-Scavenging Activity of Different Extracts

Extract / Standard	IC_{50} (µg/mL)†
Petroleum ether	298.45 ± 5.83[a]
Ethanol	52.31 ± 1.49[b]
Distilled water	128.64 ± 3.27[c]
Ascorbic acid	18.22 ± 0.76[d]

† Mean ± SD (n = 3). Different superscripts indicate significant differences (*p* < 0.05).

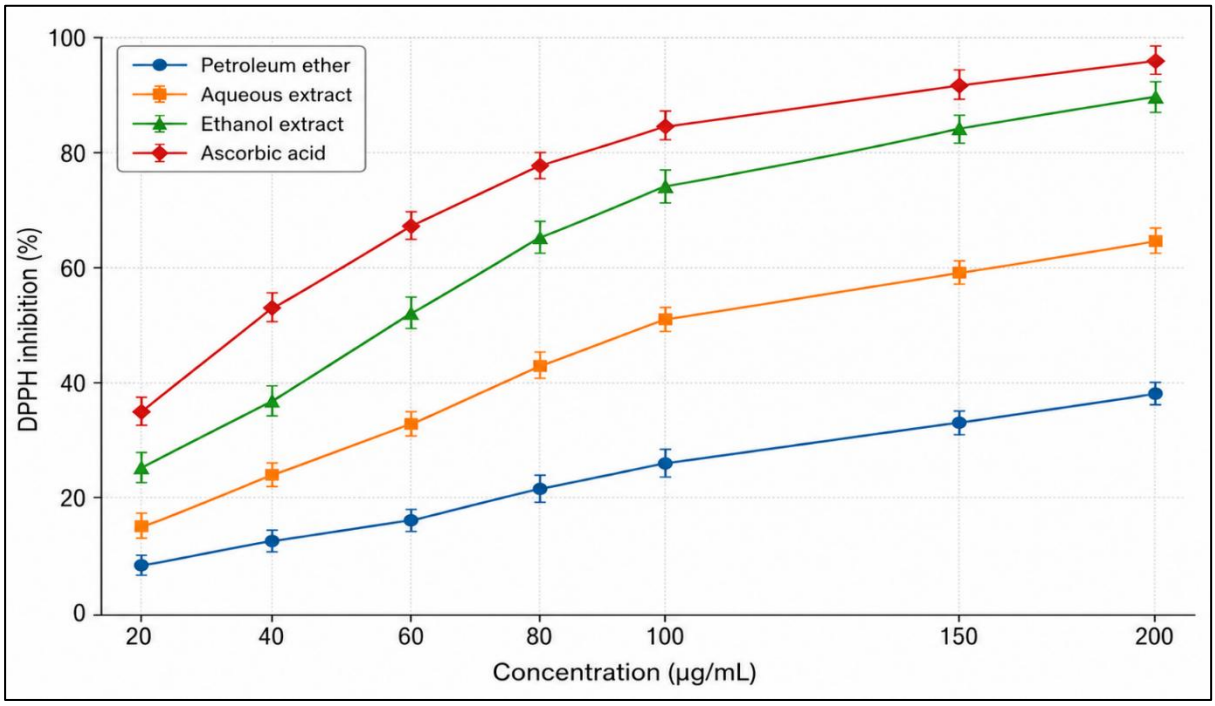

Figure 1: Concentration-Dependent DPPH Radical-Scavenging Activity

DPPH inhibition (%) is shown versus concentration (20, 40, 60, 80, 100, 150, and 200 µg/mL) on the x axis of this line graph. Four curves are displayed: aqueous extract (moderate slope, ~65% at 200 µg/mL), petroleum ether extract (shallow slope, ~38% at 200 µg/mL), ethanol extract (steep rise, ~90% at 200 µg/mL), and ascorbic acid (greatest inhibition across all points, reaching ~96% at 200 µg/mL). SD is shown by error bars.

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4.6 Antibacterial Activity

Table 6 summarizes the antibacterial activity of the three extracts as determined by the agar well diffusion technique. With significant zones of inhibition against both Gram positive and Gram negative bacteria, the ethanol extract demonstrated the widest and most potent antibacterial spectrum. The most sensitive bacteria was Staphylococcus aureus, and the ethanol extract produced the biggest zone of inhibition (19.4 ± 0.8 mm). While the petroleum ether extract exhibited zero action against Gram negative organisms and only marginal activity against Gram positive pathogens, the aqueous extract showed significant activity. Whereas the positive control (ciprofloxacin 30 µg/disc) produced zones ranging from 24.1 ± 0.5 mm for P. aeruginosa to 28.6 ± 0.7 mm for S. aureus, the negative control (10% DMSO) showed no suppression. The comparative antibacterial activity of the three extracts is shown in Figure 2.

The most powerful extract, ethanol, has its minimum inhibitory concentration (MIC) values established. The minimum inhibitory concentration (MIC) for S. aureus, B. subtilis, E. coli, and P. aeruginosa was 1.56 mg/mL, 3.125 mg/mL, 6.25 mg/mL, and 12.5 mg/mL, respectively. These results verify that Gram-positive bacteria are more vulnerable to the extract.

Table 6: Antibacterial Activity of Different Aerial-Part Extracts

Test Organism	Zone of Inhibition (mm)†	Ethanol	Distilled water	Ciprofloxacin (30 µg)
	Petroleum ether			
Staphylococcus aureus	8.2 ± 0.4^{a}	19.4 ± 0.8^{b}	12.6 ± 0.5^{c}	28.6 ± 0.7^{d}
Bacillus subtilis	7.5 ± 0.3^{a}	16.8 ± 0.6^{b}	11.1 ± 0.4^{c}	26.3 ± 0.6^{d}
Escherichia coli	6.0 ± 0.0^{a}	13.2 ± 0.5^{b}	9.8 ± 0.3^{c}	25.8 ± 0.5^{d}
Pseudomonas aeruginosa	6.0 ± 0.0^{a}	11.7 ± 0.4^{b}	8.4 ± 0.3^{c}	24.1 ± 0.5^{d}

† Mean ± SD (n = 3). Well diameter 6 mm. Means in the same row with different superscript letters differ significantly (*p* < 0.05). Ciprofloxacin was used as positive control.

(Description: A clustered bar chart with the zone of inhibition (mm) on the y axis and test organisms on the x axis. Petroleum ether, ethanol, aqueous extracts, and ciprofloxacin are shown by four bars per organism.

5. DISCUSSION

The goal of the current research was to thoroughly evaluate the phytochemical makeup, antioxidant potential, and antibacterial activity of petroleum ether, ethanol, and aqueous extracts of Tanacetum parthenium L. aerial parts. The results show that the yield of extractable matter and the range of bioactive chemicals recovered are significantly influenced by the extraction solvent selection, which in turn controls the level of biological activity.

The aqueous extract produced the largest percentage (22.14%), followed by ethanol (15.38%) and petroleum ether (4.91%). The extractive yields varied significantly. The basic idea that

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solvents with greater polarity extract more polar elements, such as simple sugars, glycosides, mucilages, and inorganic salts, is in line with this tendency [9]. The poor yield of petroleum ether extract suggests that a very small portion of the aerial biomass is made up of non-polar lipids, waxes, and chlorophylls. For other members of the Asteraceae family, similar polarity-dependent yield patterns have been documented [23].

The impact of solvent polarity on the kinds of metabolites recovered was highlighted by qualitative phytochemical screening. The capacity of ethanol to dissolve a wide range of both moderately polar and non-polar chemicals is shown by the ethanol extract's high positive results for phenolics, flavonoids, alkaloids, tannins, and terpenoids [29]. On the other hand, the aqueous extract was rich in proteins, carbohydrates, glycosides, and saponins, but the petroleum ether extract was mostly limited to terpenoids and steroids. These findings demonstrate that ethanol is an appropriate solvent for extracting a variety of bioactive secondary metabolites from T. parthenium and are consistent with the known solubility patterns of these phytochemical groups.

The greater extraction efficiency of ethanol was directly shown by quantitative measurement of the total phenolic and flavonoid contents. Compared to the water extract, the ethanol extract had 148.72 mg GAE/g of phenolics and 62.34 mg QE/g of flavonoids, which were 1.7 and 2.2 times greater, respectively. These hydrophilic polyphenols were absent from the non-polar fraction, as confirmed by the petroleum ether extract's low quantities. These findings are consistent with previous research on feverfew that found much higher polyphenol contents in hydroalcoholic extracts [21, 22]. TPC and TFC have a substantial positive connection ($r > 0.95$, data not given), indicating that flavonoids make up a significant portion of this plant's total phenolic pool.

The polyphenolic concentration was reflected in the antioxidant activity measured by the DPPH test. Compared to the water (IC • 128.64 µg/mL) and petroleum ether (IC • 298.45 µg/mL) extracts, the ethanol extract showed the lowest IC • (52.31 µg/mL), suggesting that it is a far more effective free radical scavenger. The dose-dependent scavenging shown in all extracts demonstrates that the phenolic hydroxyl groups' capacity to donate hydrogen mediates the DPPH decrease [26, 31]. Because polyphenols and flavonoids are well-known chain-breaking antioxidants that may neutralize free radicals, the ethanol extract's high polyphenol and flavonoid content is responsible for its noticeably reduced IC^{1} [6]. The ethanol extract's IC•₀ value is similar to that of numerous other medicinal plants thought to have notable antioxidant potential, despite the fact that it was less active than the pure standard ascorbic acid [8]. The aqueous extract's moderate activity is probably caused by its decreased but still noticeable phenolic load as well as potential contributions from other water-soluble reductants such reducing sugars and ascorbic acid.

The antibacterial screening demonstrated a distinct link between structure and action. The petroleum ether extract was essentially ineffective, while the ethanol extract showed the biggest

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inhibition zones and the lowest MIC values against all four test strains, followed by the aqueous extract. The distribution of phenolic and terpenoid molecules is similar to this hierarchy, indicating that the main antibacterial principles are phenolics, flavonoids, and the sesquiterpene lactone parthenolide, which is known to partition into ethanol [18,25]. Research on plant extracts often reveals that Gram positive bacteria (S. aureus and B. subtilis) are much more susceptible than Gram negative bacteria (E. coli and P. aeruginosa) [32]. Many phytochemicals are prevented from entering Gram negative bacteria by their lipopolysaccharide-rich outer membrane, which acts as a strong permeability barrier [7]. Despite being greater than that of conventional antibiotics, the ethanol extract's MIC of 1.56 mg/mL against S. aureus is regarded as significant for a crude plant extract and calls for bioassay-guided fractionation to separate the active ingredients [28].

This research has a number of strengths. A methodical comparison of extract compositions and activities was made possible by the use of a sequential solvent extraction procedure using solvents of increasing polarity. Chemical profiles and bioactivities might be correlated thanks to the combination of qualitative and quantitative phytochemical investigations. The reliability of the antibacterial data was improved by the use of standardized microbial strains and the calculation of MIC values for the most active extract.

However, there are certain limitations to the research. A more thorough antioxidant profile might be provided by other tests such ABTS, FRAP, or superoxide radical scavenging. The antioxidant assessment was based on a single assay (DPPH). There were only four bacterial strains included; it would be beneficial to test against a larger panel of clinical isolates, including resistant strains. In order to pinpoint the precise molecules causing the reported actions, the research failed to isolate and characterize individual chemicals. The results are based on plant material obtained from a particular site and season, and seasonal and geographical changes in phytochemical composition were not taken into account.

In summary, the most effective extract was found to be the ethanol extract of the aerial portions of T. parthenium, which was rich in phenolics and flavonoids and had notable antibacterial and antioxidant properties. The findings support feverfew's traditional usage and provide a scientific foundation for its further growth as a natural source of antibacterial and antioxidant compounds. To fully realize the therapeutic potential of this important medicinal plant, future research should concentrate on the separation of bioactive chemicals, investigations of synergistic interactions, and in vivo toxicological and pharmacological assessments.

6. CONCLUSION

The current study shows that the most effective extract in terms of phytochemical richness and biological activity is the ethanol extract of Tanacetum parthenium L.'s aerial parts. The ethanol extract had the greatest total phenolic content (148.72 mg GAE/g), total flavonoid content (62.34 mg QE/g), and the strongest DPPH radical-scavenging activity (IC_{50} of 52.31 µg/mL) when compared to petroleum ether and water extracts. Its higher polyphenolic content and

antioxidant capability were highly associated, supporting the idea that phenolics and flavonoids are the main agents that neutralize free radicals. With the biggest zones of inhibition and the lowest minimum inhibitory concentrations, the same extract demonstrated the widest antibacterial spectrum, especially against Gram positive Staphylococcus aureus. The impact of cell wall architecture on phytochemical penetration is highlighted by the varied sensitivity of Gram positive vs Gram negative bacteria found across extracts.

These results demonstrate the ethanol extract as a viable source of natural antioxidant and antibacterial chemicals and provide scientific support for the traditional medical usage of feverfew. The research adds to the increasing amount of data that supports the quality control and phytochemical standardization of herbal products made from T. parthenium. Nevertheless, no clinical effectiveness can be deduced since the data are limited to in vitro testing. Bioassay-guided separation and structural characterization of the active components causing the observed effects must be given top priority in future research. Before considering therapeutic use, thorough in vitro and in vivo toxicity investigations are necessary to establish safety margins. These studies will be essential to get this extract closer to a possible use as a standardized botanical product.

7. LIMITATIONS

The current research has a number of limitations that should be noted. First, because all studies were conducted using crude solvent extracts, which include complex combinations of chemicals, it is impossible to rule out the possibility of antagonistic or synergistic interactions, which might affect the bioactivities that were found. Second, only four common bacterial strains were included for the antibacterial test; a larger panel that included clinical isolates resistant to antibiotics would provide a more thorough examination of antimicrobial capability. Third, no structural characterization, compound isolation, or purification was done, and the study did not use sophisticated analytical methods like gas chromatography mass spectrometry (GC MS) or high-performance liquid chromatography (HPLC) to profile the specific phytoconstituents in charge of the activities. Fourth, the bioavailability, metabolic stability, and pharmacokinetic behavior of the active principles are still unknown since the whole study was carried out in vitro. Lastly, no assessment of acute or subacute toxicity was carried out, which is necessary to determine the extracts' safety prior to any extrapolation to in vivo models or therapeutic application. These restrictions set the course for further research on toxicity evaluation, thorough chemical fingerprinting, and bioassay-guided fractionation.

8. FUTURE SCOPE

Numerous potential research paths have a solid foundation thanks to the existing findings. Advanced chromatographic profiling using HPLC or LC MS should be used to quantify certain phenolic and flavonoid markers, such as parthenolide, luteolin, and apigenin glycosides. This will enable the chemical standardization of extracts. GC MS analysis of the petroleum ether and ethanol extracts is recommended in order to identify volatile and semi-volatile terpenoid

constituents that may contribute to the observed bioactivities. Bioassay-guided fractionation and extraction of the most active compounds are essential for identifying the specific molecules responsible for the antioxidant and antibacterial properties, which opens the door to the development of novel natural medicinal leads. Given its promising antibacterial activity against Staphylococcus aureus, an antibiofilm study is required to evaluate the extract's ability to inhibit biofilm development, a critical virulence feature in persistent infections. Cytotoxicity assays against normal mammalian cell lines should be performed in order to differentiate between particular antibacterial activity and general cellular toxicity. Additional in vivo pharmacological research using appropriate animal models is necessary to assess efficacy, pharmacokinetics, and safety in a physiological context. In order to produce a stable, safe, and effective herbal product, formulation development studies—such as topical creams, gels, or oral solid dosage forms incorporating the standardized ethanol extract—could be examined based on the strong antioxidant profile, subject to favorable toxicity outcomes.

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