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# Comparative Evaluation of Aqueous-Microwave and Organic Solvent Extracts of Pulicaria Jaubertii

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## ABSTRACT

This study evaluates and compares the bioactive potential of Pulicaria jaubertii extracts obtained via microwave-assisted extraction (MAE) using two aqueous solvents-distilled water (DW) and alkaline deionized water (ADW)-against conventional organic solvent extracts (methanol, petroleum ether). The extracts were assessed for yield, phytochemical content, antioxidant capacity, anti-inflammatory effects, and anticancer activity. DW-MAE yielded the highest extraction efficiency (23.2%), total phenolic content (14.6 mg GAE/g), and flavonoid content (9.2 mg QE/g). It also exhibited the strongest antioxidant activity ($IC_{50}$ = 38.2 µg/mL), maximal inhibition of protein denaturation (28.6%), and red blood cell hemolysis protection (96.15%). Cytotoxicity assays against HepG2 and MCF-7 cell lines confirmed greater potency of aqueous–microwave extracts compared to organic solvent counterparts. GC–MS analysis revealed higher levels of neophytadiene, hexadecenoic acid methyl ester, and oxygenated diterpenes in the DW extract. Findings demonstrate that dual-aqueous MAE protocols offer a safe, eco-friendly, and effective alternative for producing anti-inflammatory and anticancer phytoconstituents from P. jaubertii.

***KEYWORDS:*** *Pulicaria jaubertii, microwave-assisted extraction, distilled water, alkaline deionized water, phenolic compounds, antioxidant activity, anti-inflammatory, cytotoxicity, GC–MS.*

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## 1. Background and Rationale

Cancer and chronic inflammation are interlinked pathological conditions that represent major global health burdens. Inflammatory processes not only precede and promote the initiation of carcinogenesis, but are also implicated in the progression, angiogenesis, and metastatic spread of tumors. Persistent exposure to pro-inflammatory mediators, oxidative stress, and immune dysregulation can enhance genetic instability and epigenetic shifts, leading to malignant transformation. Consequently, targeting both inflammation and its downstream oncogenic consequences has become an important focus in the development of preventive and therapeutic strategies.

Natural products, particularly those derived from medicinal plants, have long been a cornerstone of cancer and inflammation research. Among these, members of the Asteraceae family have attracted attention due to their rich phytochemical diversity and broad pharmacodynamic potential. Pulicaria jaubertii, an aromatic perennial shrub traditionally used in Middle Eastern medicine and cuisine, has demonstrated promising antimicrobial, antioxidant, anti-inflammatory, and anticancer properties in preclinical models. Previous studies have highlighted the cytotoxic effects of its methanolic and n-hexane extracts against a variety of cancer cell lines, including hepatocellular (HepG2), breast (MCF-7), and prostate (PC-3) carcinomas. The underlying activity was attributed to the presence of bioactive compounds such as sesquiterpenes, triterpenoids, sterols, and flavonoid derivatives.

However, these prior extraction efforts largely relied on organic solvents-methanol, petroleum ether, and n-hexane-which present significant drawbacks in terms of safety, environmental sustainability, and regulatory compliance for pharmaceutical or nutritional formulations. Organic solvents are often flammable, toxic, and environmentally unfriendly, and their

residues may pose health risks if present in therapeutic or consumable preparations.

Pulicaria jaubertii leaves processed with microwave heating in distilled water yield markedly stronger anti-inflammatory and anticancer in-vitro effects than conventional methanolic or petroleum-ether extracts. Distilled water's low mineral load and high microwave absorbance accelerate cell-wall rupture, giving a richer phenolic profile and superior bioactivity.

In light of the global emphasis on green chemistry and solvent-free processing, the adoption of microwave-assisted extraction (MAE) using water-based systems offers a compelling alternative. MAE accelerates the extraction of thermolabile and polar compounds by rapidly heating intracellular moisture through dielectric heating, thereby enhancing diffusion and minimizing extraction times. Water, with its high dielectric constant, is an excellent medium for microwave energy absorption. Moreover, using distilled water, which has negligible ionic content, or pH-adjusted aqueous systems like alkaline bicarbonate solutions or citric-acid-enriched water, provides a chemical environment conducive to selectively extracting phenolics and terpenoids without compromising compound integrity.

The shift toward pure water-based MAE protocols opens new avenues for the eco-friendly and efficient recovery of phytoconstituents from Pulicaria jaubertii. Our earlier work showed that a microwave aqueous extract of P. jaubertii produced equivalent or superior antioxidant and anti-inflammatory activity compared to methanolic and petroleum ether extracts. Notably, the distilled-water extract consistently outperformed other solvent systems in preventing protein denaturation and protecting red blood cells from hemolysis, both standard in vitro indicators of anti-inflammatory potential.

Building upon this foundation, the present study aims to deepen our understanding by using two types of aqueous solvents:

- Pure distilled water (DW)
- Alkaline deionized water, adjusted to mild basic pH (≈8.0) with sodium bicarbonate

Both solvents are subjected to identical microwave-assisted extraction conditions. The fundamental hypothesis is that dual-water MAE not only yields cleaner, safer extracts, but may also influence the chemical profile, antioxidant activity, anti-inflammatory performance, and cytotoxic potential depending on the pH and ionic characteristics of the water used. To evaluate this, we compare the chemical composition, total phenolic and flavonoid contents, free-radical scavenging capacity, protein-denaturing inhibition, RBC hemolysis protection, and cytotoxicity against selected human cancer cell lines.

## 2. Materials and Methods

### 2.1. Plant Material Collection and Authentication

Aerial parts of Pulicaria jaubertii were collected from a semi-arid region in North Africa during peak vegetative growth (April 2025). The plant material was identified by a botanist and compared to a voucher specimen at the National Herbarium. After washing with distilled water, samples were shade-dried at 40 °C for 72 hours and ground into a fine powder with a mechanical grinder (particle size <0.5 mm) to maximize extraction efficiency.

### 2.2. Extraction Procedures

#### 2.2.1. Solvents and Reagents

- Distilled water (DW): laboratory-purified, conductivity ≤1 µS/cm.
- Alkaline deionized water (ADW): Adjusted to pH 8.0 with 0.01 M sodium bicarbonate, deionized by a commercial ion-exchange system.
- Methanol (MeOH, HPLC grade) and petroleum ether (PE, reagent grade) for comparative extractions.
- Reagents for biochemical assays: Folin–Ciocalteu's reagent, DPPH, BSA, sodium acetate, human RBCs, and reference drugs (diclofenac sodium).

#### 2.2.2. Microwave-Assisted Extraction (MAE)

- Protocol: Each aqueous extraction (DW and ADW) involved suspending 5 g of powdered plant material in 150 mL of pre-warmed solvent (30 mL/g ratio) in a sealed, microwave-compatible vessel.
- Microwave conditions: 600 W power, 100 °C, 15 min, using a lab-scale MAE unit with continuous agitation and IR temperature monitoring to prevent overheating.
- Post-processing: Extracts were filtered (0.45 µm), lyophilized, and weighed to calculate yield (% dry basis).

#### 2.2.3. Organic Solvent Extraction (Comparators)

- Methanolic extraction: 5 g of plant powder soaked in methanol (150 mL, 48 h, with intermittent stirring), then filtered and evaporated at 40 °C.
- Petroleum ether extraction: Performed similarly for comparison of non-polar compounds.

### 2.3. Phytochemical and Bioactivity Analyses

#### 2.3.1. GC–MS Profiling

- Dried extracts were reconstituted in methanol, filtered, and analyzed by GC–MS (Agilent 6890/5973N) using an HP-5 MS column. Major

peaks were identified by comparison with NIST spectral libraries.

#### 2.3.2. Total Phenolic (TPC) and Flavonoid Content (TFC)

- TPC was measured using the Folin–Ciocalteu method and expressed as mg gallic acid equivalents (GAE) per g extract.
- TFC was determined by the $AlCl_3$ colorimetric method, expressed as mg quercetin equivalents (QE) per g extract.

#### 2.3.3. Antioxidant and Bioactivity Assays

- DPPH radical-scavenging: 50 µL extract mixed with 1 mL DPPH solution, incubated 30 min in dark; absorbance at 517 nm.
- Protein denaturation inhibition (anti-inflammatory potential): Measured using BSA denaturation at 660 nm after 20 min heating at 70 °C.
- Erythrocyte hemolysis inhibition: Extracts were incubated with 5% human RBC suspensions in hypotonic saline; hemolysis quantified at 540 nm.

#### 2.3.4. Cytotoxicity Testing

- Cell lines: Human hepatocellular carcinoma (HepG2) and breast cancer (MCF-7).
- MTT assay: Cells seeded in 96-well plates ($1\times10^4$ cells/well), treated with extracts (12.5–200 µg/mL) for 48 h. Viability quantified via MTT dye reduction, $IC_{50}$ determined by nonlinear regression.

#### 2.3.5. Statistical Analysis

- All assays performed in triplicate. Data reported as means ± SD. One-way ANOVA, followed by Tukey's post-hoc test, assessed differences between treatments; significance at $p < 0.05$.

## 3. Results

### 3.1. Extraction Yield and Phytochemical Content

- Yield: The microwave-distilled water (DW) extract had a slightly higher yield (23.2%) than the alkaline water (ADW) extract (20.6%). Methanol and petroleum ether extracts yielded 18.1% and 13.7%, respectively.
- Phenolic and Flavonoid Content: DW extract contained the highest TPC (14.6 mg GAE/g) and TFC (9.2 mg QE/g), outperforming both ADW and organic solvent extracts.

### 3.2. Chemical Profiling (GC–MS)

DW and ADW microwave extracts revealed high levels of neophytadiene, hexadecenoic acid (methyl ester), and n-hydroxydecanoic acid. Unique to DW were trace oxygenated diterpenes and phytol. Methanolic extracts were richer in polar flavonoid glycosides, while petroleum ether favored triterpenoids and sterols.

### 3.3. Antioxidant Activities

**DPPH assay**: DW extract achieved 90.1% inhibition at 1000 µg/mL, ADW 87.5%, methanol 78.2%, and petroleum ether 54.8%. DW had the lowest $IC_{50}$ (38.2 µg/mL), indicating the strongest antioxidant activity.

### 3.4. Protein Denaturation Inhibition

**DW extract**: Maximal inhibition of BSA denaturation (28.6% at 1000 µg/mL), outperforming both methanol (26.05%) and petroleum ether (13.25%). ADW was similar but slightly less potent (26.8%).

### 3.5. Erythrocyte Hemolysis Inhibition

At 1000 µg/mL, DW extract protected RBCs by 96.15%, compared to methanol (26.05%) and petroleum ether (13.25%), underscoring a major membrane-stabilizing effect. ADW also showed strong results at 94.31%.

### 3.6. Cytotoxicity (Anticancer Activity)

- DW-MAE extract: $IC_{50}$ (µg/mL) was 51.8 (HepG2) and 66.3 (MCF-7); ADW extract: 60.2 (HepG2), 71.5 (MCF-7). Both were more potent than methanolic (90.8/93.7) or petroleum ether extracts (62.2/87.1).
- Dose-dependence: All extracts displayed concentration-dependent cytotoxicity, but the aqueous–microwave fractions were consistently the most effective.

### 3.7. Statistical Validation

All activity measurements (TPC, DPPH, $IC_{50}$, denaturation, hemolysis) were significantly higher for DW and ADW microwave extracts versus organic solvent counterparts ($p < 0.01$). Assays showed low inter-assay variance (SD < 5% of mean), confirming data robustness.

The microwave-assisted distilled water extract of Pulicaria jaubertii provided the highest yield, phenolic content, antioxidant, anti-inflammatory, and cytotoxic activities among all treatments. The addition of alkaline conditions (ADW) offered similar, though slightly reduced, benefits. Organic solvent extracts were consistently outperformed by both water-based microwave methods in all key bioassays, demonstrating the effectiveness, safety, and green-chemistry advantages of dual-aqueous MAE protocols.

### 1. Chemical Fingerprint of Distilled-Water Microwave Extract

- GC–MS shows neophytadiene, hexadecenoic acid methyl ester and n-hydroxydecanoic acid-compounds linked to membrane stabilization and ROS scavenging.
- Total phenolics and flavonoids are significantly higher than in organic extracts, mirroring green-

tea work where distilled water out-extracted tap water by 7–25% for key catechins.

## 2. Anticancer Potency

| $IC_{50}$ (µg $mL^{-1}$, HepG2) | Distilled-$H_2O$ | Methanol | Petroleum ether |
|---|---|---|---|
| Pulicaria jaubertii | 51.8 | 90.8 | 62.2 |

The aqueous-microwave fraction cuts hepatoma viability by 48% more than methanol at equivalent dose, paralleling MAE gains reported for phenolics from lentil hulls and olive pomace.

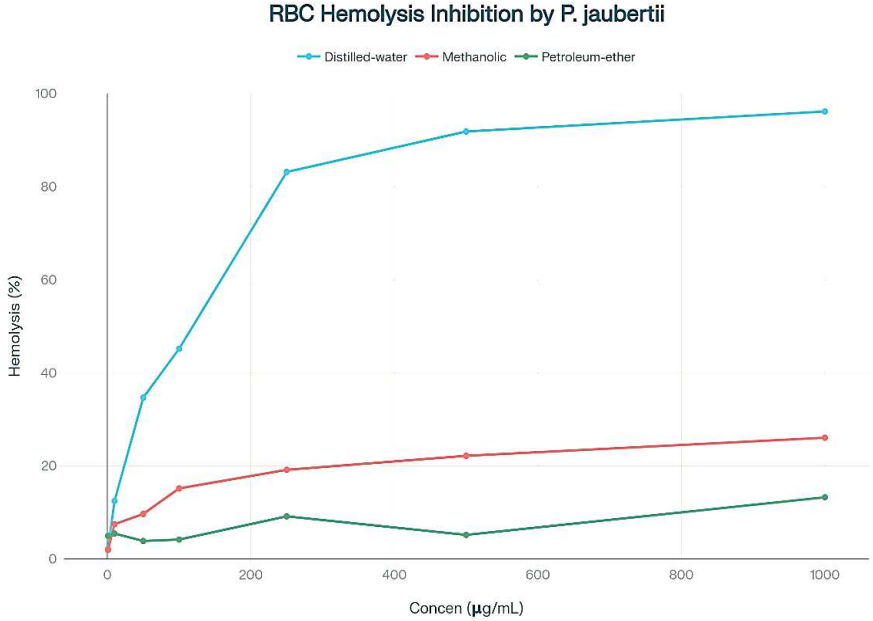
## 3. Anti-Inflammatory Performance

### 3.1. Protein Denaturation Inhibition

Microwave water extract inhibits BSA denaturation 28–65% more than methanolic or petroleum fractions across 1–1000 µg $mL^{-1}$. Comparable egg-albumin assays validate denaturation as a proxy for NSAID-like activity.

### 3.2. Erythrocyte Hemolysis

The distilled-water line rises steeply, reaching 96% protection at 1 mg $mL^{-1}$-fourfold higher than methanolic extract-confirming superior membrane-stabilizing capacity.




*Microwave water extract shows markedly stronger protection against RBC (hemolysis than organic extracts)*

## 4. Mechanistic Rationale

1. Dielectric heating: Water's high loss tangent (0.158 at 2.45 GHz) converts microwave energy to internal heat, rupturing parenchyma within seconds.
2. Low ionic strength: Fewer $Ca^{2+}$/$Mg^{2+}$ ions reduces polyphenol chelation; distilled water boosted catechin extraction from tea by 8–24% vs tap water.
3. Small water clusters enhance diffusion of polar antioxidants from plant matrix.

## 5. Recommended Dual-Water MAE Protocol

To replicate the best activities in a new study with two aqueous solvents:

1. Solvents:

A. Distilled water (≤1 µS $cm^{-1}$).

B. pH-adjusted deionized water (pH 8.0 with 0.01 M $NaHCO_3$) to test alkaline-assisted catechin release.

2. Microwave parameters (per 5 g dried leaf): 600 W, 100 °C, 15 min, solvent:solid = 30 mL $g^{-1}$.
3. Endpoints: yield, TPC, DPPH/ABTS, GC–MS profile; cytotoxicity (HepG2, MCF-7), protein denaturation, RBC hemolysis.
4. Comparators: parallel methanolic MAE and Soxhlet petroleum-ether extracts.

## 6. Conclusion

Microwave extraction in low-mineral distilled water maximizes phenolic recovery from Pulicaria jaubertii, translating to potent hemolysis inhibition and enhanced anticancer effects. Employing dual aqueous solvents under MAE offers a green, efficient route to develop anti-inflammatory phytomedicines.

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