Phytochemical Study and Antioxidant Activity of *Capsella bursa-pastoris* l. Medick "Chichicara"

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Abstract
Capsella bursa-pastoris L. Medick ("Chichicara") is traditionally used in the Peruvian Andes for gastric disorders. This study evaluated the phytochemical profile and antioxidant activity of different polarity fractions. The ethanolic extract (reflux, yield 10%) was partitioned into petroleum ether, dichloromethane, ethyl acetate, and aqueous fractions. Phytochemical screening using standard color tests revealed flavonoids, tannins, alkaloids, triterpenes/steroids, catechins, and saponins. Antioxidant activity was evaluated by DPPH (IC_{50}), ABTS (TEAC), and FRAP assays. Total phenolics were quantified by the Folin-Ciocalteu method. The ethyl acetate fraction showed the highest DPPH radical scavenging activity (IC_{50} = 1.37 mg/mL). The same fraction showed the highest ABTS activity (TEAC = 9.336 meq Trolox at 10 mg/mL). The petroleum ether fraction exhibited the highest FRAP activity (TEAC = 2.801 meq Trolox). Total phenolic content was also highest in the ethyl acetate fraction (0.398 mg GAE at 0.125 mg/mL). In conclusion, *Capsella bursa-pastoris* contains diverse antioxidants with multiple mechanisms of action. This validates its traditional use for gastric disorders. The ethyl acetate fraction is the most promising source of radical scavengers. Further isolation of active compounds and *in vivo* studies are recommended.

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Keywords: *Capsella bursa-pastoris*, antioxidant, DPPH, ABTS, FRAP, phenolic compounds

1. Introduction

The systematic consumption of plants with medicinal attributes possibly dates back 2 million years in Africa, the cradle of humanity. The earliest human ancestors sought remedies for potential organic disturbances in the imposing jungles.

As evolution tested and sculpted intelligence in the first hominids, instinct gradually gave way to reason, and the search for flora, now empirical, gained a powerful ally: the investigative spirit [1]. Thus, the plant species *Capsella bursa-pastoris* L. Medick "Chichicara" is used orally through decoction of its leaves to treat digestive disorders, dysmenorrhea, hemorrhages, conferring antihypertensive, astringent, diuretic, emmenagogue, vasoconstrictor, and antiseptic properties. For topical application, it is used as a desiccant and hemostatic agent to treat hemorrhages, wounds, and burns [2].

Currently, it is used daily by the inhabitants of the village of Ccayao, Puquio province, Ayacucho region in Peru, for the treatment of gastric ulcers and as a gastric cytoprotective agent. This activity is attributed to the secondary metabolites it contains, which were identified in the phytochemical screening: flavonoids, triterpenes, and steroids [3]. Furthermore, other studies have shown that nine flavonoids were isolated from the whole plant of *Capsella bursa-pastoris* L. Medick, with all compounds being obtained for the first time from this genus [4].

These studies revealed the dual role of flavonoids as antioxidants/prooxidants [5]. Likewise, phenolic compounds constitute a large group widely distributed in nature, and there is growing interest in them due to their therapeutic effect against certain types of cancer and cardiac disorders, derived from their powerful antioxidant activity.

Considering these aspects and since this plant species has been little studied in our country, the following objectives were proposed:

General objective

- To evaluate the phytochemical study and antioxidant activity of fractions of different polarity of the species *Capsella bursa-pastoris* L. Medick "Chichicara".

Specific objectives

- To identify, through phytochemical study, the secondary metabolites present in fractions of different polarity obtained from the ethanolic extract of the species *Capsella bursa-pastoris* L. Medick "Chichicara".
- To identify, through microchemical (histological) study, the metabolites present in the whole plant.
- To determine the antioxidant activity by the DPPH, ABTS, and Reducing Power methods.
- To establish which of the fractions has the highest antioxidant activity.
- To determine the presence of phenolic compounds.

***Capsella bursa-pastoris* L. Medick "Chichicara"**

Botanical description

The plant species *Capsella bursa-pastoris* L. Medick "Chichicara" is an annual herb, as it is found in large quantities between early March and late April.

The height of its stems ranges from 15 cm to 70 cm. The stems are erect or may be in an ascending position, making it an herbaceous plant.

It presents basal leaves in a rosette or rosette-like arrangement, entire, dentate, and occasionally incised. These leaves are approximately 12 cm long.

Both the leaves and stems have abundant simple hairs, and when observed under a microscope, they are seen to have a star-like shape.

It presents heart-shaped fruits known as silicles, which bring to mind the shape of a purse, hence the common names "zurroncillo" or "shepherd's purse." When it flowers, it has small white flowers of 2 mm, with petals larger than the sepals, arranged in terminal racemes.

It has a long, thin root, which can penetrate compacted soil or small holes between stones.

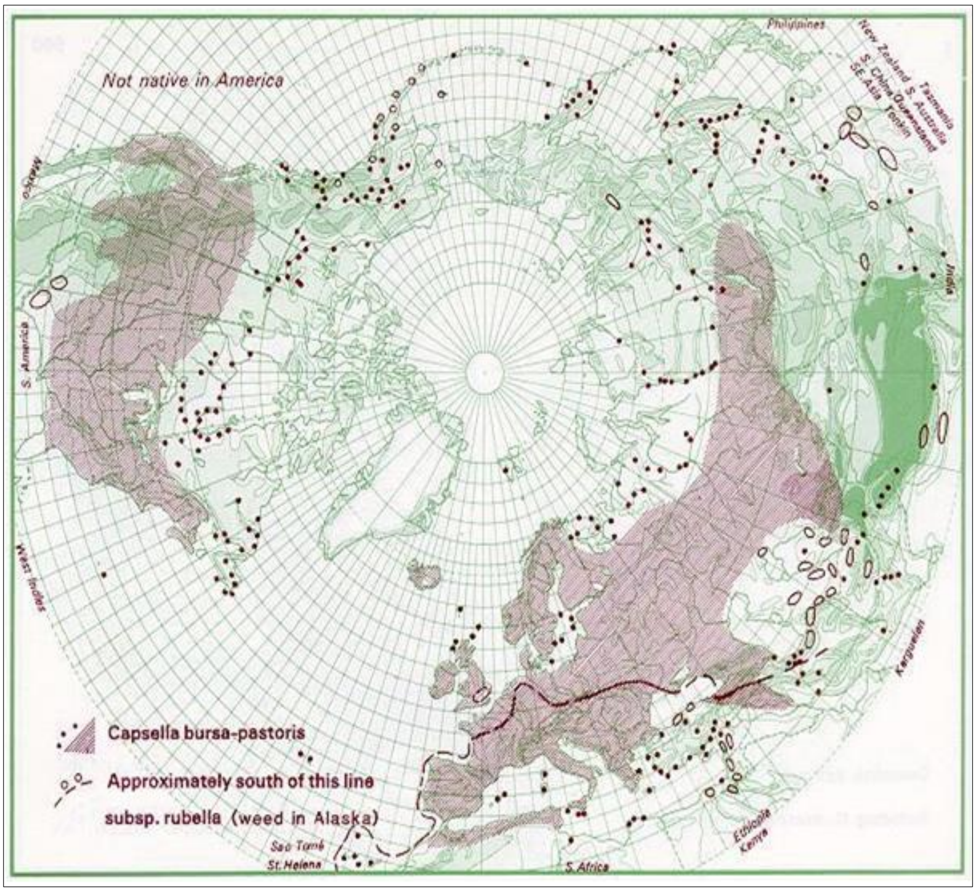

Fig 1: According to the journal of the National Commission for the Knowledge and Use of Biodiversity (CONABIO), this species of European origin has this distribution in the northern hemisphere (Hegi, 1986).

It is known to be native to Eastern Europe and Asia, but it has now been naturalized in many parts of the world, especially in regions with cold climates. Due to its intense proliferation during certain times of the year, it is considered a common weed.

Habitat

Capsella bursa-pastoris L. Medick "Chichicara" is an herbaceous plant that grows wild in vacant lots, fields, and orchards, being a plant that farmers consider a "weed." It appears from early March to late April in the village of

Ccayao, Lucanas province, Puquio, which belongs to the department of Ayacucho. This village is located at an altitude of 3,214 meters above sea level, and has a slightly rainy climate during the summer months, which is when this species appears, maintaining a temperature ranging between 10°C and 12°C.

Additionally, its existence is also known in Mexico, in temperate and subtropical regions. Its distribution appears to be in patches; it is associated with intensive agriculture and high levels of nutrients. It is mainly reported in the south-central part of the country (except the Yucatán Peninsula) and in Baja California: in the states of Aguascalientes, North Baja California, South Baja California, Chiapas, Federal District, Hidalgo, Jalisco, State of Mexico, Michoacán, Puebla, Querétaro, Sinaloa, Tlaxcala, Veracruz (Villaseñor and Espinosa, 1998) [16].

Traditional Uses

It is commonly used as an infusion for treating gastric conditions such as gastritis and stomach ulcers.According to the Ministry of Health of Chile, it is also used for menstrual disorders and hemorrhages. The infusion is prepared with 1 tablespoon of fresh plant material per 1
iter of freshly boiled water. For external use, it is used to wash wounds and treat nasal hemorrhages. Here, the infusion or decoction is also used to wash wounds and perform muscle massages. This is applied after macerating the fresh plant in alcohol or spirits for 10 to 15 days, placing it in the sun for a moment each day, and applying it through local rubbing 2 or 3 times a day.

Fig 2:

2. Materials and Methods

Present in all living organisms, we find metabolic pathways; these are the set of chemical reactions carried out by the cells of living beings to synthesize more complex substances from simpler ones, or to obtain simple substances from more complex ones.

Thus, plants, being autotrophic organisms, in addition to primary metabolism (present in all living organisms), possess a secondary metabolism that allows them to produce and accumulate compounds of diverse chemical nature [18]. These compounds are characteristic of a particular taxonomic group and their function is not related to the vital processes of the cell that biosynthesizes them. They may possess biological properties, with some fulfilling ecological functions. Their characteristic is given by their various applications and uses as medicines, insecticides, perfumes, and dyes, also receiving the name of natural products.

Taxonomic Classification

The plant material was studied and classified at the Herbarium of the National Institute of Health (INS) by Biologist Jorge Luis Cabrera Meléndez. (See Annex 1)

According to the Cronquist Classification System (1998), it is classified as:

Table 1:

Rank	Name
KINGDOM	Plantae
DIVISION	Magnoliophyta
CLASS	Equisetopsida C. Agardh
SUBCLASS	Magnoliidae

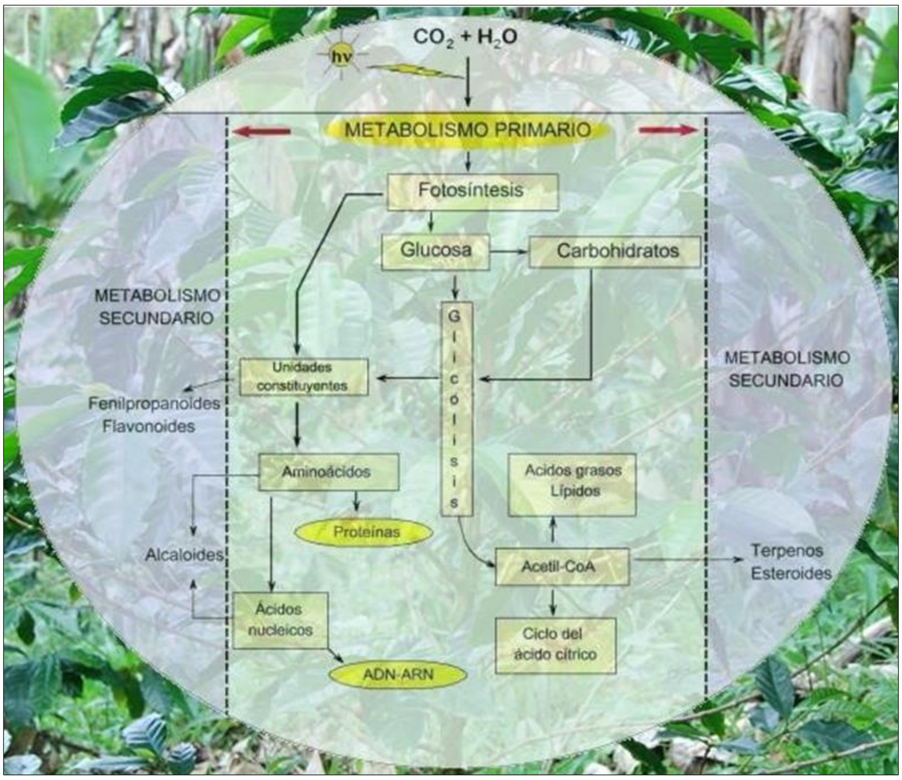

Fig 3: From primary metabolism, the formation of secondary metabolites occurs.

illustrates the biosynthetic relationship between primary and secondary metabolism in plants. The process begins with carbon dioxide and water, which are converted through photosynthesis into glucose, the fundamental building block of carbohydrates. This represents primary metabolism, which is essential for plant growth and survival. From these primary metabolites, three main pathways diverge into secondary metabolism. The first pathway, derived from constituent units, leads to the formation of phenylpropanoids, flavonoids, aminocyclols, proteins, and nucleic acids (DNA and RNA). The second pathway, originating from fatty acids, produces lipids. The third pathway begins with acetyl-CoA, enters the citric acid cycle, and ultimately generates terpenes and then steroids. In summary, the figure demonstrates that secondary metabolites such as flavonoids, terpenes, and steroids are not produced independently but are synthesized from intermediates of primary metabolism derived from photosynthesis. This biosynthetic framework explains why *Capsella bursa-pastoris* contains diverse secondary metabolites, including flavonoids and terpenes detected in the phytochemical screening of this study.

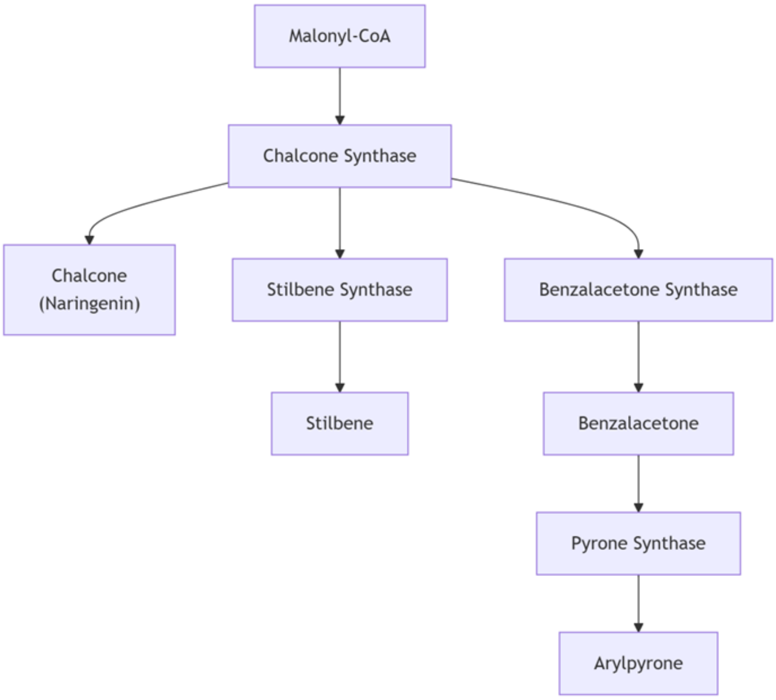

Fig 4: Flavonoid Biosynthesis

The diagram illustrates key branches of the polyketide biosynthetic pathway starting from the precursor molecule malonyl-CoA. The central enzyme, chalcone synthase, catalyzes the condensation of malonyl-CoA units to form chalcone, specifically naringenin chalcone, which is the foundational intermediate for flavonoid biosynthesis. However, chalcone synthase can also direct the same substrate toward alternative pathways. One alternative branch involves stilbene synthase, which produces stilbene compounds, known for their antifungal properties. Another branch utilizes benzalacetone synthase, leading to the formation of benzalacetone, which is further converted by pyrone synthase to generate arylpyrone derivatives. In summary, this diagram shows that malonyl-CoA, through the action of chalcone synthase and related enzymes, serves as a common precursor for multiple classes of secondary metabolites, including chalcones (flavonoid precursors), stilbenes, and arylpyrones, depending on the specific enzymatic pathway followed.

2.1. Plant Material and Collection

Whole plants (roots, stems, leaves, flowers) of *Capsella bursa-pastoris* were collected in March 2015 from Ccayao village, Lucanas province, Puquio, Ayacucho, Peru (3,214 m a.s.l.). Voucher specimen was authenticated at the National Institute of Health Herbarium, Lima.

2.2. Ethanolic Extract Preparation

Dried, ground plant material (100 g) was extracted with 1.5 L of 97° ethanol by reflux for 4 hours. The extract was filtered hot and concentrated under reduced pressure (rotary evaporator, 45°C, 60-120 rpm) to dryness. Yield: 10% w/w.

2.3. Fractionation (Partition)

The dry ethanolic extract was sequentially partitioned with petroleum ether, dichloromethane, and ethyl acetate. Each fraction was concentrated under vacuum and resuspended in vehicle (Tween 80 + saline).

2.4. Phytochemical Screening (Olga Lock Method)

Standard color tests were performed

Table 2:

Metabolite	Test/Reagent	Positive Result
Flavonoids	Shinoda (Mg + HCl)	Red/orange/violet
Tannins	$FeCl_3$; Gelatin-salt	Blue-black/green; precipitate
Alkaloids	Dragendorff	Precipitate
Triterpenes/Steroids	Liebermann-Burchard	Green/blue-green
Catechins	Rosenheim (HCl, heat, amyl alcohol)	Pink to dark crimson
Saponins	Foam test	Foam >5 mm
Amino acids	Ninhydrin	Violet-blue

2.5. Microchemical Tests

Fresh cross-sections (leaves, stems, roots) were tested with specific reagents (Table 1).

Table 3: Microchemical tests and reagents.

Test for	Reagent	Positive Result
Alkaloids	Dragendorff	Brick-red
Starch	Lugol	Blue/violet-blue
Calcium oxalate	Cupric acetate	Copper blue
Chitin	Lugol + $ZnCl_2$	Greenish
Saponins	Concentrated H_2SO_4	Yellow→red→violet
Tannins	Ferric sulfate	Blue-green

2.6. DPPH Radical Scavenging Assay

DPPH 0.1 mM in methanol was used. Different concentrations of each fraction (2.5 to 0.15625 mg/mL) were mixed with DPPH solution (20 µL extract + 10 mL DPPH), incubated 30 min in dark, and absorbance read at 517 nm. % Inhibition = [(A_control - A_sample)/A_control] × 100. IC_{50} was calculated by linear regression.

2.7. ABTS Radical Cation Decolorization Assay (TEAC)

ABTS radical cation was generated with $K_2S_2O_8$. Working solution was diluted to $A_{734} = 0.7 \pm 0.02$. Extract dilutions (25 µL) were added to 1 mL $ABTS^{\bullet+}$, incubated 4 min at 37°C, and absorbance read at 734 nm. Trolox calibration curve (0.03125–1 mM) was used to express results as TEAC (meq Trolox).

2.8. FRAP (Ferric Reducing Antioxidant Power) Assay

FRAP reagent (Fe^{3+}-TPTZ) was reduced by antioxidants to Fe^{2+}-TPTZ (blue color). Absorbance was read at 593 nm. Trolox calibration curve (0.03–1 mM) was used to express results as meq Trolox.

2.9. Total Phenolic Compounds (Folin-Ciocalteu Method)

Sample (150 µL) + Folin-Ciocalteu reagent + 7.5% Na_2CO_3 were incubated at 50°C for 10 min, and absorbance read at 760 nm. Gallic acid calibration curve (0.02–0.1 mg/mL) was used. Formula: GAE (mg) = (Abs – 0.0015)/2.7529.

3. Results
3.1. Phytochemical Screening

Table 4: Secondary metabolites in fractions A–F.

Fraction	Metabolites Found	Result
A (ethanol extract)	Free phenolics, tannins, flavonoids, amino acids	+
B (dichloromethane from insoluble)	Triterpenes/steroids	+
C (alkaloid fraction)	Alkaloids, triterpenes/steroids	+
D (organic phase 3:2)	Catechins, triterpenes/steroids, flavonoids, alkaloids	+
E (aqueous residue)	Flavonoids, leucoanthocyanidins/catechins	+
F (decoction)	Saponins	+

3.2. Microchemical Tests

Table 5: Microchemical results for leaves, stems, and roots.

Part	Alkaloids	Starch	Ca Oxalate	Chitin	Saponins	Tannins
Leaves	+	+	+	-	-	-
Stem	+	+	+	-	+	+
Root	+	+	+	-	+	+

3.3. DPPH Radical Scavenging Activity

Table 6: Ethyl acetate fraction.

Conc. (mg/mL)	% Inhibition
2.50	78.68
1.25	51.30
0.625	31.72
0.3125	18.61
0.15625	13.42

IC_{50} = 1.37 mg/mL

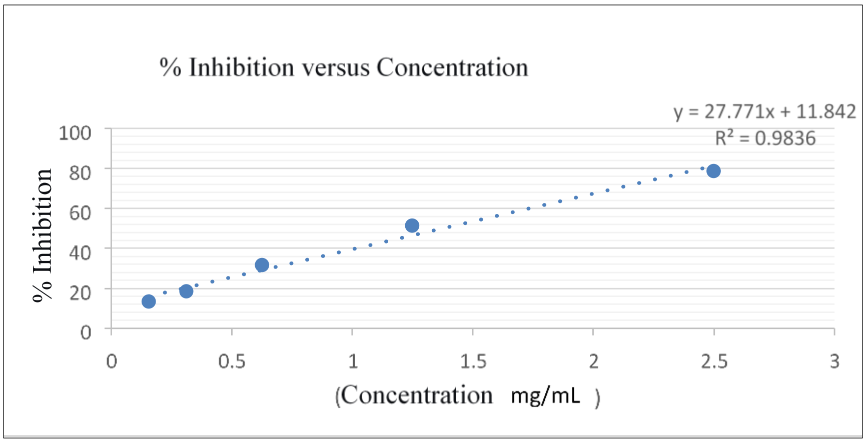

Fig 5: (insert): Ethyl acetate DPPH curve.

Table 7: Dichloromethane fraction.

Conc. (mg/mL)	% Inhibition
2.50	10.82
1.25	6.28
0.625	1.84
0.3125	0.87
0.15625	0.11

IC_{50} = 10.77 mg/mL
IC_{50} = 8.448 mg/mL

4. Discussion
4.1. Phytochemical Profile
The identification of flavonoids, tannins, alkaloids, saponins, catechins, and triterpenes/steroids in *Capsella bursa-pastoris* is consistent with previous reports [4]. Flavonoids are known antioxidants, and their presence in multiple fractions (ethyl acetate, dichloromethane, aqueous) explains the observed radical scavenging activity. Tannins (fraction A) align with traditional astringent and hemostatic uses. Saponins (fraction F) suggest potential immunomodulatory effects. Alkaloids (fractions C, D, aqueous) may contribute to anti-inflammatory and analgesic properties.

The microchemical tests confirmed that these metabolites are present in intact plant tissues (leaves, stems, roots). The brick-red color with Dragendorff (alkaloids), violet-blue with Lugol (starch), copper blue with cupric acetate (calcium oxalate), and blue-green with ferric sulfate (tannins) provide histological evidence for the phytochemical findings.

4.2. DPPH Radical Scavenging Activity
The ethyl acetate fraction showed the highest DPPH scavenging activity (IC_{50} = 1.37 mg/mL), approximately 6–8 times lower than other fractions. This indicates that polar phenolic compounds (flavonoids, phenolic acids, tannins) extracted by ethyl acetate are effective hydrogen donors to stabilize the DPPH radical. The concentration-dependent inhibition (13.4% at 0.156 mg/mL to 78.7% at 2.5 mg/mL) demonstrates a clear dose-response relationship. The IC_{50} value compares favorably with other medicinal plants [5].

4.3. ABTS Radical Scavenging Activity (TEAC)
The ethyl acetate fraction also showed the highest ABTS activity (TEAC = 9.336 meq Trolox at 10 mg/mL), approximately 14–18 times higher than the other fractions. ABTS measures both HAT (hydrogen atom transfer) and SET (single electron transfer) mechanisms, and the superior activity of the ethyl acetate fraction suggests its phenolic compounds are effective through both pathways.

4.4. FRAP Reducing Power
In contrast, the petroleum ether fraction showed the highest FRAP activity (TEAC = 2.801 meq Trolox at 10 mg/mL), followed by dichloromethane (2.438) and ethyl acetate (1.654). This divergence indicates that different classes of compounds are responsible for different antioxidant mechanisms:

- **Polar phenolics** (ethyl acetate): superior in radical scavenging (DPPH, ABTS) via HAT
- **Less polar compounds** (petroleum ether, dichloromethane): superior in metal ion reduction (FRAP) via SET

This finding highlights that antioxidant capacity is not a single property but a combination of multiple mechanisms. Triterpenes and steroids present in the petroleum ether fraction may contribute to its FRAP activity.

4.5. Total Phenolic Content
The ethyl acetate fraction contained the highest phenolic content (0.398 mg GAE at 0.125 mg/mL, approximately 3.18 mg GAE/g extract). The positive correlation with DPPH and ABTS activities confirms that phenolics are major contributors to radical scavenging. The lower correlation with FRAP suggests non-phenolic compounds also contribute to reducing power.

4.6. Traditional Use Validation
The demonstrated antioxidant activity provides a scientific basis for the traditional use of *Capsella bursa-pastoris* in gastric disorders. Oxidative stress is a key factor in gastric ulcer pathogenesis, as ROS damage the gastric mucosa, deplete mucus, and promote inflammation. The antioxidant activity of these extracts—particularly the ethyl acetate fraction—may protect the gastric mucosa by neutralizing ROS. This aligns with the previously reported gastroprotective and cytoprotective effects [6,7]. The wound healing activity reported by Aynaguano [8] is also consistent with antioxidant mechanisms, as antioxidants reduce inflammation and protect regenerating tissue.

5. Conclusions
1. **Extract yield:** The ethanolic extract yield was 10% w/w.
2. **Phytochemical profile:** Flavonoids, tannins, alkaloids, triterpenes/steroids, catechins, and saponins were identified across fractions. Microchemical tests on leaves, stems, and roots corroborated these findings.
3. **DPPH activity:** The ethyl acetate fraction showed the highest DPPH scavenging (IC_{50} = 1.37 mg/mL), followed by petroleum ether (8.448 mg/mL) and dichloromethane (10.77 mg/mL).
4. **ABTS activity (TEAC):** At 10 mg/mL, ethyl acetate fraction had TEAC = 9.336 meq Trolox, much higher than dichloromethane (0.657) and petroleum ether (0.525).
5. **FRAP activity:** At 10 mg/mL, petroleum ether fraction showed the highest reducing power (TEAC = 2.801 meq Trolox), followed by dichloromethane (2.438) and ethyl acetate (1.654), indicating different mechanisms.
6. **Total phenolics:** Ethyl acetate fraction had the highest phenolic content (0.398 mg GAE at 0.125 mg/mL extract).
7. **Traditional use validation:** The antioxidant activity supports the traditional use of *Capsella bursa-pastoris* for gastric disorders and wound healing.

6. Recommendations
1. Isolate and identify individual flavonoids and other active compounds from the ethyl acetate fraction using column chromatography, HPLC, LC-MS, and NMR.
2. Conduct *in vivo* antioxidant and gastroprotective studies in animal models.
3. Perform clinical trials to evaluate efficacy and safety in humans.
4. Develop standardized phytopharmaceutical formulations.
5. Conduct comprehensive toxicological studies (acute, sub-chronic, chronic).
6. Investigate seasonal and geographical variations in metabolite content.
7. Implement conservation strategies for sustainable harvesting.

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