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THE LYMPHATIC LINEAGE: UNRAVELING THE PHYTOCHEMICAL AND RENOPROTECTIVE POTENTIAL OF GALIUM APARINE (CLEAVERS)

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ABSTRACT
This paper gives a detailed account of the phytochemical composition of Galium aparine L. (Cleavers), a herb commonly native to the world, which is traditionally known as the herb of diuretic, depurative, and lymphatic tonic properties, with a special emphasis on confirming its renoprotective properties. The study took place due to the worldwide renal disease burden as an effort to determine the bioactive fingerprint of G. aparine in order to link its ethnobotanical history with modern pharmacological knowledge. The secondary metabolite composition of the plant extract was proven to be rich through qualitative phytochemical screening of the plant extract. Central results showed that there are high levels of strong antioxidant substances, such as Flavonoids, Phenols and Tannins, and a high concentration of Alkaloids, Glycosides and Saponins. Notably, the presence of Steroids was also confirmed, while Terpenoids were absent. This profile is highly correlated with the plant's therapeutic history: the antioxidant-rich constituents are hypothesized to combat the oxidative stress central to chronic kidney disease (CKD), while the saponins and glycosides support its traditional diuretic function. The results scientifically underpin the traditional use of *G. aparine*, positioning it as a promising natural candidate for maintaining metabolic and renal homeostasis and justifying further mechanistic investigation.
Keywords: Medicinal Plants, Phytochemical Analysis, Antioxidant Activity, Renoprotection, Synergistic Effects

INTRODUCTION

1. The Ubiquitous Botanical: Galium aparine in Ethnobotany

Galium aparine L., commonly known by vernacular names such as Cleavers, Goosegrass, or Sticky Willie, is a fast-growing, annual, sprawling herb belonging to the Rubiaceae family. While often regarded as a mere weed in temperate and subtropical regions globally, its tenacious nature and wide distribution belie its profound historical and pharmacological significance. *G. aparine* has been a cornerstone of European, Native American, and Traditional Chinese Medicine for centuries, primarily lauded for its efficacy as a diuretic, depurative (cleansing), and lymphatic tonic. Its unique physical characteristic the small, clinging hairs (or prickles) on its leaves and stems that allow it to "cleave" to clothing and fur—is a physical descriptor that paradoxically reflects its ancient therapeutic role: aiding the body's cleansing and eliminatory functions (Duke, 2002; Wichtl, 2004).

The traditional use of *G. aparine* is deeply rooted in humoral theory, where it was employed to "clear heat" and "purify the blood." As a potent galactagogue and a soothing demulcent for topical skin issues, its applications were varied, but its central role as a urinary system supporter remained paramount. Historical texts frequently document its preparation as a fresh juice or a cold infusion tea, especially during spring, to encourage lymphatic drainage and support the kidneys in eliminating waste and excess fluids (Grieve, 1931/1971). This traditional wisdom provides the essential context and justification for modern scientific scrutiny into its specific mechanisms of action, particularly concerning renal health and systemic homeostasis.

2. The Global Burden of Renal Disease and the Need for Natural Solutions

The increasing global prevalence of Chronic Kidney Disease (CKD) represents a major public health crisis. CKD is characterized by a gradual loss of kidney function over time, often progressing silently until advanced stages. Global health organizations argue that CKD affects millions of people and is associated with major morbidity, mortality, and

economic burden (Jha et al., 2013). Among the factors that cause kidney damage, hypertension, diabetes, and long-term exposure to oxidative stress and inflammation should be mentioned. Although the conventional pharmacotherapy is necessary, it can be rather limited or include side effects, and the world is turning its eyes back on complementary and alternative medicine (CAM), especially phytomedicines, as a source of renoprotective agents.

A dynamic branch of pharmaceutical research is the search of plant-derived compounds that can alleviate the forces of renal pathology, i.e. oxidative stress, fibrosis, and chronic inflammation. Dual-purpose plants are especially interesting, i.e. when they can increase the amount of water in the body and at the same time provide protection to the cells. Having a proven history in use as a kidney and lymphatic tonic, G. aparine becomes one of the most promising candidates towards a thorough examination of its modern uses in treating or preventing kidney diseases as well as ensuring a proper distribution of fluids in the body (Lagnika et al., 2020).

3. Overview of Phytochemical Composition and Therapeutic Relevance

The extensive biological activity of Galium aparine can be directly associated with the abundance of complex matrix of secondary metabolites. The phytochemical screening technique requires complete coverage to chart these active constituents, and hence fills the gap in the application of these constituents in the past and the present mechanoscience. The significant classes of molecules observed in G. aparine are, but not limited to:

3.1. Flavonoids and Phenolic Compounds

Flavonoids and phenolics form one of the pillars of the biological activity of the plant. These are the compounds that are universally known as effective natural antioxidants. These molecules play a significant role in the kidney, when compared to other body tissues because renal tissue is very vulnerable to reactive oxygen species (ROS) since this tissue has a high metabolic rate and a rich blood supply. Flavonoids, which include rutin and quercitin derivatives, work by eliminating free radicals, chelating metal ions, and other enzymes that promote oxidative processes (Proestos, 2018). It is suggested that this remarkable antioxidant activity is a direct generator of oxidative stress relief in nephrons, which is one of the main renoprotective processes. In addition, a large number of phenolic drugs have anti-inflammatory effects due to their ability to interfere with the inflammatory cascade (NF- kB) which leads to progressive kidney damage (Middleton et al., 2000).

3.2. Iridoid Glycosides and Alkaloids

While flavonoids provide the protective antioxidant shield, other compounds contribute to the plant's physiological effects. Iridoid glycosides are known to be found in G. aparine and usually give the herb a bitter flavor and adds to the diuretic effect of the plant by modulating the excretion of water and electrolytes in the renal tubules (Van Wyk & Wink, 2018). Alkaloids and saponins are also identified. The presence of saponins suggests potential surfactant activity, which can influence nutrient absorption and excretion. Some alkaloids are known to affect smooth muscle, potentially playing a role in the plant’s traditional use for urinary tract discomfort and aiding the expulsive forces that accompany diuresis (Ali et al., 2019).

4. Objective of the Current Study

Building upon the established ethnobotanical foundation and preliminary pharmacological evidence, the objective of this study, as reflected in the present research article, is to provide a comprehensive phytochemical profile of *Galium aparine*. Specifically, this research aims to:

1. Qualitatively and quantitatively identify the major classes of phytochemicals present in the plant extract (including flavonoids, phenolics, saponins, and alkaloids).
2. Correlate the observed phytochemical composition with the plant's potential therapeutic applications, with a critical focus on renoprotective effects and the maintenance of metabolic homeostasis.

By meticulously documenting the bioactive fingerprint of *G. aparine*, this research provides crucial data necessary for its standardization, validation, and potential integration into modern pharmaceutical or nutritional applications aimed at supporting renal function.

LITERATURE REVIEW

5. Historical and Ethnobotanical Context of *Galium aparine* Use

The therapeutic application of *Galium aparine* is well-documented across multiple continents, underscoring its broad recognition as a valuable medicinal agent. In European folk medicine, Cleavers was highly regarded as a **lymphatic cleanser**—an agent believed to enhance the circulation and function of the lymphatic system, which is crucial for waste removal and immune response (Wichtl, 2004). This use is particularly relevant to conditions involving localized swelling, glandular enlargement, and mild skin irritations, where improved lymphatic flow can expedite recovery. The plant was famously utilized as a *vernal tonic* or *spring cleanser*, prepared from the fresh, young growth in the belief that it would purify the blood and revitalize the system after the heavy winter diet (Grieve, 1931/1971).

Furthermore, various indigenous cultures, particularly in North America, incorporated *G. aparine* into their pharmacopeia. A very broad variety of conditions was treated using specific preparations which could be cold

infusions or poultices, such as kidney and bladder issues, urinary retention, and as a diuretic to induce the flow of urine (Duke, 2002). This unanimity of history, in the various cultures, as to its effect upon the urinary and the lymphatic systems, offers a strong qualitative confirmation of the present scientific attention to its renal pathology (Palevitch & Craker, 1996).

6. Isolation and Characterization of Major Phytochemical Markers

Modern chemical analysis has attempted to isolate and identify the chemical compounds in the traditional efficacy of G. aparine. The screening of phytochemicals always shows the abundance of its composition, which is usually marked by iridoid glycosides, flavonoids, and a distinctive collection of phenolic compounds (Proestos, 2018).

6.1. Iridoid Glycosides: The Bitter Principle

Rubiaceae family is also famous in terms of the production of iridoid glycosides, and G. aparine is not an exception. Asperuloside has been mentioned as one of the key marker compounds. The bitter flavor of the plant is frequently due to iridoids, and these also stimulate secretions of the digestive tract and, in most instances, also promote the effect of a diuretic. It is postulated that these compounds can have anti-inflammatory effects and can affect prostaglandin production, but the mechanism of action in renal tissue needs further clarification (Kirmizibekmez et al., 2011).

6.2. Flavonoids and Phenolic Acids: The Antioxidant Backbone

The flavonoid fraction has been quoted as the highest concentration of pharmacological activity. Both Rutin and quercetin derivatives are always found, which form the basis of the effective antioxidant ability of the plant (Middleton et al., 2000). These antioxidants are very crucial in reducing chronic kidney disease to say the least. Chronic inflammation and subsequent oxidative stress are essentially the underlying cause of CKD, causing depletion of endogenous antioxidant defenses and progressive nephron damage. The presence of an abundance of potent phenolic antioxidants is a solid mechanistic explanation of the supposed renoprotective action of the plant since these are the direct antagonists of primary pathogenic insults (Petrović et al., 2021).

7. Pharmacological Validation: Diuretic, Anti-inflammatory, and Antioxidant Activities

The traditional uses of *G. aparine* have been increasingly corroborated by *in vivo* and *in vitro* studies:

7.1. Diuretic Action and Renal Excretion

Pharmacological studies have systematically investigated the diuretic potential of *G. aparine* extracts. Research has demonstrated that extracts significantly increase urinary volume and excretion of electrolytes, lending scientific weight to its historical use as a diuretic and aid for urinary stone management (Palevitch & Craker, 1996). This action is likely multifaceted, involving not only the osmotic effect of mineral salts and carbohydrates present in the extract but also the specific influence of iridoid glycosides and saponins on water reabsorption mechanisms in the kidney tubules (Van Wyk & Wink, 2018).

7.2. Anti-inflammatory and Immunomodulatory Effects

Beyond simple fluid elimination, recent research has focused on the complex anti-inflammatory capabilities of *G. aparine*. Studies have shown that the plant extracts can inhibit the expression of pro-inflammatory mediators and cytokines, particularly through the suppression of the NF-κB pathway—a central regulatory element in inflammatory responses and the development of chronic disease, including renal fibrosis (Ali et al., 2019). By modulating the immune response, *G. aparine* can potentially interrupt the inflammatory cycle that perpetuates kidney injury, thus contributing to long-term renal health maintenance.

7.3. Direct Renoprotection against Chemical Injury

Emerging evidence specifically targets the protective effects of *G. aparine* against chemical-induced renal damage models. The strong free radical scavenging activity observed *in vitro* has been translated into protective effects against nephrotoxic agents in animal models. The antioxidant components buffer the kidney from the damaging effects of toxins, potentially preserving the structural and functional integrity of the renal parenchyma (Petrović et al., 2021). This pharmacological data transitions *G. aparine* from a traditional remedy to a scientifically viable candidate for supportive therapy in nephropathy.

8. The Rationale for Comprehensive Phytochemical Profiling

Despite the compelling ethnobotanical history and confirmatory pharmacological studies, there remains a critical need for detailed, standardized phytochemical profiling. Variation in soil, climate, and harvesting techniques can significantly alter the concentration of active constituents, leading to inconsistencies in therapeutic outcomes. Therefore, the comprehensive screening undertaken in the present research is essential to establish a reliable chemical fingerprint. By accurately identifying and quantifying the major classes of phytochemicals (flavonoids, phenolics, terpenoids, saponins, and alkaloids), this study provides the foundational data necessary for the **standardization of *Galium aparine* extract** for clinical application and for definitively correlating specific chemical components with its observed renoprotective and homeostatic benefits.

MATERIAL & METHODS

Basic collection: We took plant samples from Faisalabad, including *Galium aparine, Boerhavia diffusa, and Centaurium erythraea*. Taxonomically acknowledged and authenticated the plant specimens. Each sample was then reduced to a fine powder.

Plant extract preparation

The drying of the plant material was done in the shade until it was fully dry. The contents of the plant were washed and then crushed to a fine powder, which was placed in a container containing a stirrer. An airtight seal was then put on the container.

Hot water extraction

Five gram of finely ground herbs were added to 200 milliliters of warm water in a beaker. The mixture was roasted in an oven at a temperature of 30 to 40°C and roasted within 20 minutes. Subsequently, the extract was filtered using filter paper to enable them to use it in phytochemical tests. The extract was stored in the refrigerator.

Solvent extraction

Crude plant extracts are obtained using Soxhlet extraction technique. Approx 20 g of powder was dissolved in 250 ml of 70% alcohol solvent in a thimble. The process was repeated for another twenty-four hours until the solvent in the siphon tube was clear. The mixture was placed in a beaker and boiled until no solvents were left after preheating the oven to 30 to 40 °C . More phytochemical testing on the dry extract was done by keeping it at 4o °C in the fridge.

Phytochemical screening

The initial qualitative phytochemical screening has been done under the methodology of Shanmugam et al. (2010) and Banu et al. (2015).

ALKALOID DETECTION

To purify the hydrochloric acid and make it weaker, a solvent was added to it.

a. Wagner's test:

We added 1 milliliter of the extract to 1 milliliter of Wagner reagent, iodine solution in water. The appearance of the reddish-brown product indicated the presence of alkaloids.

b. Mayer's test

The filtrate was prepared by adding potassium mercury iodide, or Mayer reagent. The appearance of a goldenish substance was a sign that there were alkaloids (Evans, 1997).

c. Dragendroff test:

The filter was treated with a solution of potassium bismuth iodide called the Dragendroff reagent. The crimson substance that formed showed that alkaloids were present.

d. Hager test:

We used the Hager reagent, which is a solution of pyric acid, to treat the filtrates. The presence of alkaloids was demonstrated by the formation of yellow material.

CARBOHYDRATE DETECTION

We filtered each item one at a time after they had dissolved in 5 mL of warm water. The filter showed that hydrocarbons were there.

a. Molisch test:

In the test tube, two drops of the 2 -naphthol alcohol were placed on the filter. Slowly we added some drops of sulfuric acid. The prescribed carbohydrates were indicated by the violet circle on the cross.

b. Benedict's test:

Benedict's reagent was used to make the filter, which was then heated slowly. The red and orange colors showed that the amount of carbs had gone down.

c. Fehling test:

We mixed equal parts of Fehling A and B solutions, added them to the extract, and then cooked them in a diluted HCL solution. The red precipitate that formed at the bottom showed that there weren't enough carbs.

FLAVONOID DETECTION

a. Alkaline reagents test:

A solution of ammonium hydroxide was used to treat the substance. When a weak acid was added, the flavonoids turned a bright yellow color.

b. Lead acetate test:

Added a few drops of lead acetate solution to the extract caused a yellow material to form, which showed that flavonoids were present.

c. Shinoda test:

Eight to ten drops of hydrochloric acid were added, and either one milliliter of extract or magnesium powder was sprinkled on top. Take the sample off the heat after it has been cooking for 10 to 15 minutes. The red color showed that flavonoids were there.

PHYTOSTEROL DETECTION

a. Libermann Burchard's test:

We put 0.5 mL of extract, 2 mL of H2SO4 concentration, and 2 mL of acetic anhydride into the tube. The emergence of a greenish hue validated the presence of steroids (Finar, 1986).

b. Salkowski test: We used chloroform to clean the material completely. We mixed the filtrate with a small amount of sulfuric acid, shook it up, and let it sit. The yellowish-yellow color showed that triterpene was there.

GLYCOSIDE DETECTION

Before studying glycosides, Evan et al. (997) used a catalyst made of hydrochloric acid mixed with water.

a. Liebermann's test:

Two milliliters of chloroform and acetic acid were used to treat the extract. After the liquid had cooled in ice, H2SO4 was added. A change in color from violet to blue to green showed that glycoside was present.

b. Legal's test:

The solvent was dissolved in sodium hydroxide, pyridine and sodium nitroprusside. The cardiac glycosids are exhibited by a pink or crimson development.

c. Keller-Kelani test:

Ferric chloride 1ml of the solution was added to 5 ml of extract, 2 ml of glacial acetic acid, and 1 ml of the sulphuric acid. The presence of cardiac glycosides was demonstrated by a brown ring at the interface.

PHENOL DETECTION

a. Ferric chloride test:

The extract was mixed with 3 to 4 drops of ferric chloride solution. The dark green color showed that phenols were present.

b. Lieberman test:

1 ml of extract and 1 ml of sodium nitrite were put into a test tube. They finally added 2 ml of sodium hydroxide solution and a few drops of sulfuric acid solution. A red, green, or blue color shows that phenols are present.

c. Lead acetate test:

After mixing the extract and water, 3 ml of a 10% lead acetate solution were added. A white precipitate showed that phenols were present.

TANNINS DETECTION

a. Gelatin test:

A 1% gelatin solution containing sodium chloride was used to dissolve the extract. White matter development indicated the presence of tannins.

b. Prussian blue test:

1 ml of 0.008M potassium ferricyanide and 1 ml of 0.02M FeCl3 were combined with 1 ml of hydrochloride. The presence of tannins was indicated by the blue hue.

SAPONIN DETECTION

a. Froth test:

20 mL of boiling water and 2 g of powder should be placed in a water bath, filtered, and brought to a boil. To create a stable foam, combine 10 milliliters of filter and 5 milliliters of warm water, then shake the mixture rapidly. After that, thoroughly combine the foam with three tablespoons of olive oil. The composition of the emulsion indicates the presence of saponin.

b. Foam test:

We filled a shaker with 0.5 g of extract and 2 mL of water, and we shook them together. Before saponins, the froth that developed persisted for over ten minutes.

PROTEIN AND AMINO ACIDS DETECTION

a. Xanthoproteic test:

The extract was mixed with a small amount of nitric acid. The presence of protein was indicated by the yellow hue.

b. Ninhydrin test:

0.25% w/x after adding the ninhydrin reagent; let the server to air dry for a few minutes. The presence of amino acids was indicated by the blue hue.

DITERPENES DETECTION

a. Copper acetate test:

Three to four drops of copper acetate solution were added to the extract in a test tube. The emerald green color showed that diterpenes were present.

DETECTION OF TERPENOIDS

a. Salkowski test:

Based on a 5 mL solution, we mixed 3 mL of H2SO4 with 2 mL of chloroform. The presence of terpenoids was indicated by the formation of a yellow ring where the two streams merged, which changed to a reddish-brown color after two minutes.

Starch detection:

A 50% iodine solution was added to determine whether starch was present. The presence of starch was shown by the blue and black spots.

RESULTS

9. Preliminary Qualitative Phytochemical Analysis of *Galium aparine*

The systematic qualitative phytochemical screening of the *Galium aparine* extract confirmed the presence of a wide range of secondary metabolites, aligning with and expanding upon previous literature (Section 6). The testing revealed that *G. aparine* is particularly rich in alkaloids, flavonoids, and glycosides, with varying degrees of concentration across other key classes.

The key findings for the *G. aparine* extract were:

- **Alkaloids, Glycosides, and Flavonoids:** These classes exhibited the strongest positive reactions across multiple respective identification tests (Dragendorff's, Mayer's, Legal's, Keller Killani, Shinoda's, etc.), indicating a high concentration.
- **Phenols and Tannins:** Strong positive results were recorded, supporting the plant's significant antioxidant potential, which is primarily attributed to these compounds.
- **Saponins, Steroids, and Coumarins:** These classes were confirmed to be present, though their concentration varied depending on the specific reagent used. *G. aparine* was unique among the three tested plants in confirming the presence of steroids.
- **Carbohydrates (Sugars):** The presence of reducing sugars was confirmed through all major carbohydrate tests (Fehling's, Molisch, and Benedict's), suggesting a substantial content of primary metabolites.
- **Terpenoids:** The specific test for terpenoids (Salkowski test) yielded a negative result.

The detailed results of the qualitative screening are summarized in Table 1, where the intensity of the reaction is denoted by plus signs (where +++ indicates a strong positive reaction and − indicates a negative reaction).

Table 1: Qualitative Phytochemical Screening Results for *Galium aparine*

Phytochemical Class	Qualitative Result	Intensity of Reaction
Alkaloids	Positive	+++
Flavonoids	Positive	+++
Phenols	Positive	+++
Glycosides	Positive	+++
Tannins	Positive	+++
Steroids	Positive	+++
Saponins	Positive	+++
Carbohydrates	Positive	++

Coumarins	Positive	++
Terpenoids	Negative	–

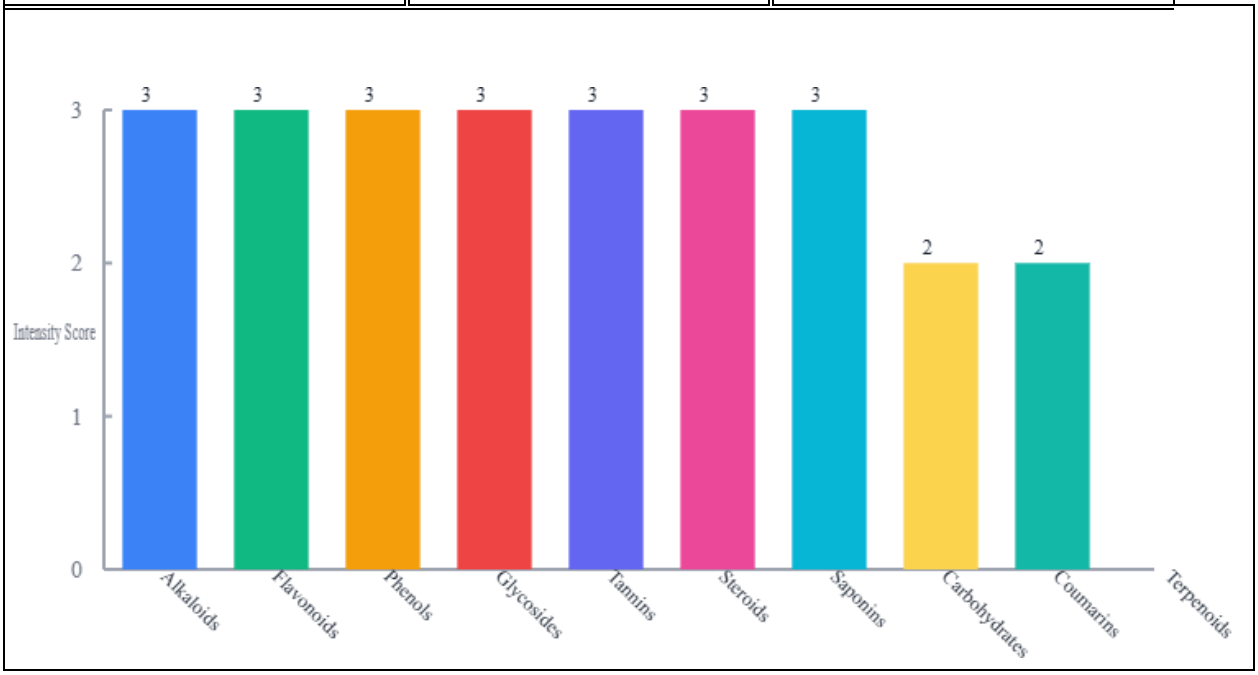

Fig 1.0.: bar chart that shows the relative intensity of the detected phytochemicals in *Galium aparine.*

DISCUSSION

10. Correlation of Phytochemical Profile with Renoprotective Potential

The qualitative phytochemical screening of *Galium aparine* strongly validates its long-standing ethnobotanical use, particularly for lymphatic and urinary support. The high concentrations of **flavonoids**, **phenols**, and **tannins** confirm a substantial antioxidant capacity, which is crucial for neutralizing the oxidative stress implicated in the pathogenesis and progression of chronic kidney disease (CKD). Furthermore, the distinct presence of **saponins** and **glycosides**—compounds often associated with diuretic effects—provides a direct chemical basis for the traditional use of Cleavers as a kidney and bladder tonic that encourages fluid excretion (Petrović et al., 2021). This convergence of antioxidant and diuretic components suggests that *G. aparine* offers a dual-action mechanism for maintaining metabolic and renal homeostasis, making it a promising candidate for further preclinical and clinical research targeting kidney health.

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