Qualitative Phytochemical Profile of Five Different Crude Solvents of *Galium asperifolium* Wall

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Abstract

The purpose of this work was to conduct a qualitative phytochemical screening of *Galium asperifolium* Wall extracts, including aqueous, ethanol, methanol, n-hexane, and ethyl acetate extracts, to identify potential bioactive compounds. The presence of tannins, flavonoids, phenols, terpenoids, saponins, anthocyanins, cardiac glycosides, coumarins, and alkaloids was detected, indicating their possible involvement in the plant's medicinal properties. Further research is necessary to explore the pharmacological potential of these compounds, isolate them, and characterize their bioactive properties responsible for the observed medicinal effects.

Keywords: *Galium asperifolium*, qualitative phytochemical screening, potential bioactive compounds.

1.0 Introduction

Medicinal plants have long been recognized for their abundance of biologically active compounds possessing therapeutic properties. Since ancient times, these plants have been widely used across the globe to treat different kinds of health problems, including asthma, gastrointestinal problems, urinary and respiratory problems [1-3]. Currently, there is a emerging demand for more plant-based drugs, as they are considered safer when compared to modern synthetic medicines. The pharmacological importance of these plants lies in the chemical natural compounds they contain,

which can produce beneficial physiological effects on the human body. Moreover, due to the vast array of chemical compounds synthesized by plants, they are capable for the development and discovery of new therapeutic agents [4]. Phytochemical compounds, also known as secondary metabolites, are widely recognized for their diverse biological activities [5]. These properties include free radical scavenging activity, antimicrobial effects, anticancer potential, and stimulation of the immune system and reduction of platelet aggregation. Within the plant system, there are over a thousand known phytochemicals, with many more yet to be discovered. While it has been established that plants produce these phytochemicals as a means of self-protection, recent scientific findings suggest that these compounds can also provide protective benefits to humans against various diseases [6]. Medicinal plants of the *Galium* genus has been utilized in medicine in past for treating wounds, ulcers, acne, and skin issues. *Galium setaceum* exhibits sedative, diuretic, antibacterial, antitumor, and antioxidant properties [7]. *Galium verum* is used in various cultures for conditions like hepatitis, skin infections, gout, and epilepsy [8]. *Galium tricornutum* subsp. *Longipedunculatum* is traditionally utilized as a remedy for the infection of skin and as a diuretic for kidney disorders in folk medicine [9]. *Galium aparine*, commonly known as catch weed bedstraw, is utilized for its therapeutic properties in treating skin conditions such as seborrhea [8]. *Galium asperfolium* is a perennial herb with branched stems and scabrid or glabrous angles. It has whorls of 6-8 leaves, measuring 2-5 mm, and many-flowered inflorescences with white corollas (3.5 mm) and glabrous or granulate fruit (2 mm). The purpose of this research was to examine the phytochemicals present in the aerial parts of *Galium asperifolium* by analyzing its aqueous, ethanolic, methanolic, n-hexane and ethyl acetate extracts.

2.0 Materials and Methods

2.1 Collection of Plant Materials

The plant *Galium asperifolium* (**Fig. 1**) *belongs* to the family *Rubiaceae* was collected from the Nathiagali Galiyat, district Abbottabad, KPK, Pakistan. The identification of the plant was facilitated through the reference of Pakistan's botanical catalog. The collected specimens were washed with tap water and shade dried for 21 days. The dried plant was finely powdered using an electrical grinder and then stored in a sealed container for future use.

Fig. 1: *Galium asperifolium* Wall.

2.2 Preparation of Plant Extracts for Phytochemical Screening

Approximately 10 grams of dried *Galium asperifolium* powder was soaked in 100ml of various solvents (distilled water, ethanol, MeOH, ethyl acetate, and n-hexane) inside reagent bottles. These mixtures were left at room temperature for 12 days, being shaken twice daily. After the designated period, the extracts were filtered using Whatman filter paper No.1. Subsequently, the filtrates were subjected to evaporation under reduced pressure and then dried using a rotary evaporator at 55°C. The resulting dried extracts were then carefully stored in properly labeled bottles in a refrigerator at 5°C until they were needed for further use.

2.3 Qualitative Phytochemical screening of Secondary Metabolites

The plant extracts were introduced to standard screening methods to confirm the occurrence of Alkaloids, Carbohydrates, Sterols, Tannins, Saponins, Flavonoids, Quinones, Cardiac glycosides, Anthocyanin, Terpenoids, Phenols, Couramins, Protein, Phlobatannins, Anthraquinone, and Glycosides.

2.3.1 Test for Alkaloids

2.3.1.1 Wagner’s Test

A few drops of Wagner's reagent were added to 2-3 ml of the extract. The occurrence of alkaloids is confirmed by the formation of a reddish-brown precipitate [10].

2.3.1.2 Mayer's Test

To 2ml of plant extract, a 2 to 3 drops of Mayer's reagent and 2ml of concentrated HCl were added. A positive confirmation of the test was indicated by the formation of creamy to greenish precipitates [11].

2.3.1.3 Hager's Test

Upon introducing a small quantity of Hager's reagent to 2 ml of the plant extract, distinct yellowish-colored precipitates emerged. This occurrence serves as a definitive confirmation of the presence of alkaloids within the extract [12].

2.3.2 Test for Carbohydrates

2.3.2.1 Benedict's Test

After subjecting 2 ml of plant extract to gentle heating in a water bath, following the addition of a small quantity of Benedict's reagent, a notable formation of reddish-brown precipitate became evident. This observation strongly indicated the potential presence of carbohydrates within the tested extract [13].

2.3.2.2 Fehling's Test

In a test tube, a balanced combination of Fehling's A, comprising $CuSO_4$ dissolved in distilled water, and Fehling's B, containing Potassium tartrate and NaOH in deionized water, was thoroughly blended. Subsequently, a small quantity of plant extract was carefully introduced into the amalgam, which was then subjected to boiling. The emergence of distinct brick-red precipitates within the mixture provided clear evidence of the presence of reducing sugars in the examined sample [14].

2.3.2.3 Molisch's Test

To a test tube containing 2 ml of plant extract, a small quantity of Molisch's reagent was meticulously added. Subsequently, 0.2 ml of concentrated H_2SO_4 was introduced slowly through the sides of the test tube. The emergence of a distinctive purple to violet ring at the junction of the liquids provided definitive confirmation of the presence of carbohydrates in the analyzed plant extract [13].

2.3.3 Test for Proteins

2.3.3.1 Millon's Test

By adding a small quantity of Millon's reagent to 2 ml of the plant extract solution, the appearance of white-colored precipitates was observed. This unmistakable occurrence serves as clear evidence of the presence of proteins within the analyzed sample [15].

2.3.3.2 Xanthoproteic Test

To 2 ml of the plant extract, a small number of concentrated nitric acid drops were carefully introduced. The manifestation of a distinctive yellow color served as a significant indicator of the presence of protein within the analyzed extract [16].

2.3.4 Test for Saponins

2.3.4.1 Foam Test

In a graduated cylinder, 1 ml of the extract solution was meticulously mixed with 20 ml of distilled water. The resulting mixture was vigorously agitated for a duration of 15 minutes. The presence of saponins was discerned by the formation of a lasting froth that persisted after the agitation had ceased [17].

2.3.4.2 Lead acetate Test

By introducing a 1% lead acetate solution to 1 ml of the solution of plant extract, the emergence of white precipitates became evident. This distinctive occurrence provided a clear indication of the presence of saponins within the tested extract [18].

2.3.5 Test for Tannins

2.3.5.1 Ferric Chloride Test

To the solution 1 ml of plant extract, 2 ml of a 5% $FeCl_3$ solution was introduced. The appearance of a dark green color confirm the occurrence of tannins [18].

2.3.2 Gelatin Test

Within a test tube, a combination of 2 ml of plant extract and 2 ml of a 1% solution of Gelatin, along with NaCl, was skillfully mixed. As a result of this amalgamation, the appearance of a distinct white precipitate became noticeable. This outcome strongly implies the potential presence of tannins within the tested plant extract [19].

2.3.5.3 Lead acetate Test

By carefully introducing a few drops of a 10% lead acetate solution to 5 ml of the plant extract, the emergence of a yellow or red precipitate was observed. This change in color serves as conclusive evidence of the presence of tannins within the examined extract.

2.3.6 Test for Flavonoids

2.3.6.1 Alkali reagent Test

By introducing a few drops of sodium hydroxide solution to one milliliter of the plant extract, the emergence of yellow to red precipitates was observed. This distinct alteration in color serves as a significant indicator of the presence of flavonoids within the analyzed extract [19].

2.3.6.2 Shinoda's Test

Minute fragments of magnesium ribbon were introduced to the extract solution along with concentrated hydrochloric acid, added drop by drop in a cautious manner. After a brief duration, the emergence of colors ranging from pink, scarlet, crimson red, to even green to blue was noted. These distinct color changes serve as indicative markers of the potential presence of flavonoids within the tested extract [20].

2.3.6.3 Acid Test

To 2 ml of the extract, a 2 to 3 drops of dilute sulfuric acid were introduced. The observation of an orange color signifies the existence of flavonoids [11].

2.3.7 Test for Glycosides

2.3.7.1 Killaer kilani Test

Within 2 ml of the extract, a mixture of glacial CH_3COOH, a single drop 5% solution of $FeCl_3$, and concentrated H_2SO_4 was meticulously combined. The occurrence of a distinct reddish-brown color at the junction of the two liquid layers, accompanied by a bluish-green upper layer, is a compelling signal of the presence of glycosides within the tested extract [10].

2.3.8 Test for Quinone

2.3.8.1 Sulfuric Acid Test

To 1 ml of the plant's extract, 1 ml of concentrated H_2SO_4 was introduced. The appearance of a red color serves as confirmation for the presence of quinone [13].

2.3.9 Test for Coumarins

1 ml of plant extract was combined with 1 ml of a 10% sodium chloride (NaCl) solution. The occurrence of a yellow color indicates the presence of coumarins in the extract [21].

2.3.10 Test for Pholobatannins

By introducing a few drops of 2% hydrochloric acid (HCl) to 1 mille liter of the plant extract, the appearance of a distinct red color became evident. This conspicuous change in color serves as a reliable indicator of the potential presence of phlobatannins within the analyzed plant extract [22].

2.3.11 Test for Anthraquinone

In order to determine the presence of anthraquinone, 1 ml of plant extract was mixed with a 10% ammonia (NH_3) solution and then shaken. The development of a pink color serves as an indicator of the presence of anthraquinone [13].

2.3.12 Test for Terpenoids

2.3.12.1 Salkowski's test

A combination of 5 ml of plant extract and 2 ml of chloroform was skillfully mixed. Subsequently, concentrated sulfuric acid was added drop by drop with careful consideration. The manifestation of a distinctive red-brown color following this reaction provides a clear indication of the presence of terpenoids within the analyzed extract. [23].

2.3.13 Test for Anthocyanin

To 2 ml of the plant extract, a small amount of concentrated sulfuric acid was introduced. The emergence of a yellowish-orange color signifies the presence of Anthocyanin [12].

2.3.14 Test for Phenols

2.3.14.1 Ferric Chloride Test

By combining 1 ml of the plant extract with 2 ml of distilled water and introducing a few drops of 10% $FeCl_3$, a noticeable transformation occurred. The appearance of a discernible blue or green color serves as a reliable indicator of the potential presence of phenols within the tested plant extract [24].

3.0 Results and Discussion

Plants are a rich source of diverse phytochemical compounds, which have been extensively studied for their potential health benefits [10]. In this study, the qualitative phytochemical screening of *Galium asperifolium* Wall was carried out using different solvents, and the results revealed the presence of various bioactive compounds. Plants possess a wide array of phytochemical compounds, including alkaloids, phenolic acids, vitamins, tannins, terpenoids, lignins, stilbenes, flavonoids, betalains, coumarins, amines, quinones, and other metabolites, which exhibit potent antioxidant activity [13]. Numerous studies have demonstrated that these antioxidants have diverse beneficial effects, such as antibacterial, antitumor, antimutagenic, anti-inflammatory, antiatherosclerotic, anticarcinogenic, antifungal, and antiviral properties [17]. The consumption of natural antioxidants has been associated with a reduced risk of conditions like diabetes, cancer, cardiovascular diseases, and other age-related ailments [19]. Natural products have been an integral part of traditional plant based medicines throughout history [18]. Phytochemicals can be extracted from various plant parts, including flowers, bark, leaves, and seeds, as each part may contain dynamic components [23]. Understanding the chemical constituents of plants is valuable, as this information is essential for synthesizing complex chemical substances [24]. Various

researchers have reported the phytochemical screening of different medicinal plants [12, 13, 15, 21, 22]. The findings from this study add to the growing body of knowledge about the phytochemical profile of medicinal plants and their potential applications in medicine and healthcare.

In the present study the examination of phytochemicals in *Galium asperifolium* Wall. Was carried out using different solvents such as ethanol, distilled water, methanol, ethyl acetate, and n-hexane. The quantitative phytochemical analysis of the aerial parts of *Galium asperifolium* indicated the presence of alkaloids, carbohydrates, tannins, and proteins in the ethanolic, methanolic, aqueous, ethyl acetate, and n-hexane extracts of the plant. Saponins and flavonoids were not detected in the methanolic extract but were present in the ethanolic, aqueous, $CH_3CO_2CH_2CH_3$, and n-hexane extracts. Terpenoids were found in the aqueous, ethanolic, and n-hexane extracts, while they were absent in the methanolic and ethyl acetate extracts. Anthraquinone was absent in all the extracts. Quinones and coumarins were not present in the methanolic extract but were found in the ethanolic, aqueous, ethyl acetate, and n-hexane extracts. Phenols were not detected in the ethanolic and methanolic extracts of the plant. Cardiac glycosides were found in the methanolic and n-hexane extracts. Anthocyanin was absent in the methanolic extract of the plant. Phlobatannins were only present in the ethyl acetate extract .The summarized results are provided in **Fig. 2** and **Table-1**.

The phytochemical analysis of *Galium asperifolium* using different solvents revealed a diverse array of bioactive compounds present in the plant's aerial parts. The choice of solvent significantly influenced the phytochemical profile, highlighting the importance of considering solvent selection when investigating specific classes of compounds. These findings contribute valuable insights into the medicinal potential of *Galium asperifolium* and open up opportunities for further research on the isolated compounds' individual bioactivities and potential therapeutic applications.

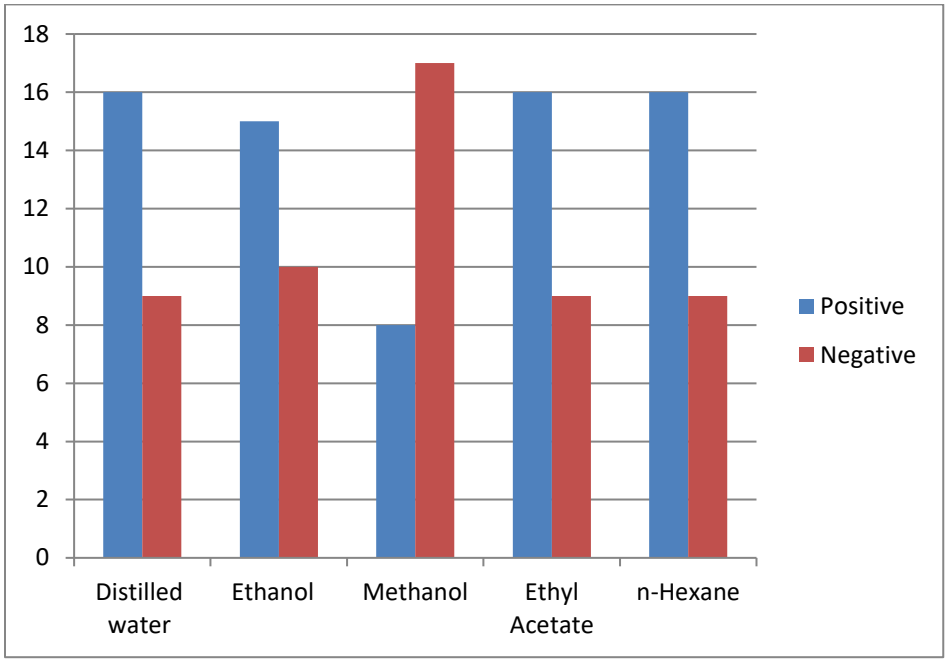

Fig. 2: Total positive and negative results of phytochemicals in 5 different solvents

Table-1: Preliminary Phytochemical screening of the five different extract of aerial parts of *Galium asperifolium* Wall.

S.NO	CONSTITUENTS	TESTS	DISTILLED WATER	ETHANOL	METHANOL	ETHYL ACETATE	N-HEXANE
1	**Alkaloids**	Wagner's test	+	-	-	-	+
		Mayer's test	-	+	+	+	+
		Hager's test	+	+	+	+	+
2	**Carbohydrates**	Benedict's test	+	+	+	+	+
		Molish's test	-	-	+	+	+
		Fehling's test	+	+	+	+	-
3	**Saponins**	Foam test	+	+	-	-	+
		Lead acetate test	-	-	-	+	-
4	**Proteins**	Millon's test	+	-	-	+	+
		Xanthoproteic test	+	+	+	-	-
5	**Flavonoids**	Alkali reagent test	+	+	-	+	+
		Acid test	+	+	-	-	-
		Shinoda's test	+	+	-	-	-

6	**Tannins**	Ferric chloride test	-	+	+	+	-
		Gelatin test	-	-	-	+	+
		Lead acetate test	+	+	-	-	+
7	**Terpenoids**	Salkowski's test	+	+	-	-	+
8	**Phenols**	Ferric chloride test	+	-	-	+	+
9	**Quinones**	Sulfuric acid test	+	+	-	+	+
10	**Cardaic glycosides**	Ferric chloride test	-	-	+	-	+
11	**Anthocyanin**	Sulfuric acid test	+	+	-	+	+
12	**Glycosides**	Keller-killiani test	-	-	-	+	-
13	**Anthraquinones**	Borntrager's test	-	-	-	-	-
14	**Coumarins**	NaOH test	+	+	-	+	
15	**Phlobatannins**	HCl test	-	-	-	+	-

4.0 Conclusion

Medicinal plants are known to possess abundant secondary metabolites, making them highly valued in traditional medicine for their effectiveness in treating various ailments. Their anti-inflammatory, antispasmodic, analgesic, and diuretic properties are attributed to the presence of high levels of alkaloids, phenols, tannins, and flavonoids. Based on the findings of the current investigation, it can be inferred that *Galium asperifolium* exhibits significant medicinal activities. The phytochemical screening study confirms the presence of pharmacologically active compounds in *Galium asperifolium*, including alkaloids, carbohydrates, proteins, terpenoids, flavonoids, phenols, saponins, and tannins. Further isolation of the active constituents and subsequent in-vivo or in vitro studies are necessary to validate the traditional use of this plant.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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References

1. Cousins, D. and M.A. Huffman, *Medicinal properties in the diet of gorillas: an ethno-pharmacological evaluation.* African study monographs, 2002. **23**(2): p. 65-89.
2. Tian, X.-R., et al., *Three new glycosides from the whole plant of Clematis lasiandra Maxim and their cytotoxicity.* Phytochemistry Letters, 2014. **10**: p. 168-172.
3. Shahid, M.S., et al., *Phytochemical group test and pharmacological investigations of Persicaria barbata (L.) H. Hara.* Phytopharmacology Research Journal, 2023. **2**(1): p. 1-15.
4. McChesney, J.D., S.K. Venkataraman, and J.T. Henri, *Plant natural products: back to the future or into extinction?* Phytochemistry, 2007. **68**(14): p. 2015-2022.
5. Hussain, M., et al., *Antimicrobial and Cytotoxic Potential of Anemone tetrasepala Royle.* Phytopharmacology Research Journal, 2023. **2**(1): p. 41-48.
6. Rao, B.N., *Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention.* Asia Pacific Journal of clinical nutrition, 2003. **12**(1).
7. Jan, A.K., et al., *Composition of the essential oil of Galium setaceum.* Chemistry of Natural Compounds, 2018. **54**: p. 586-587.
8. Layali, I., M.A. Ebrahimzadeh, and M. Joulaei, *Antioxidant properties of Galium verum.* International Journal of Life Science and Pharma Research, 2016. **6**(3): p. L31-L37.
9. Jan, A.K., et al., *In vitro antifungal and antibacterial activities of extracts of Galium tricornutum subsp. longipedunculatum.* Journal of Enzyme Inhibition and Medicinal Chemistry, 2009. **24**(1): p. 192-196.
10. Kokate, C., A. Purohit, and S. Gokhale, *Carbohydrate and derived Products, drugs containing glycosides, drugs containing tannins, lipids and protein alkaloids.* Text book of Pharmacognosy, 2001. **7**: p. 133-166.
11. Ramesh, B., *Phytochemical screening in n-hexane/ethyl acetate extract of Thespesia populnea (L.) Stem bark.* International Journal, 2016. **14**: p. 1272-1284.
12. Tiwari, P., et al., *Phytochemical screening and extraction: a review.* Internationale pharmaceutica sciencia, 2011. **1**(1): p. 98-106.
13. Evans, W., *Plants in medicine: the origins of pharmacognosy.* Trease and Evans Pharmacognosy, 2009: p. 467-469.
14. Somkuwar, D.O. and V.A. Kamble, *Phytochemical screening of ethanolic extracts of stem, leaves, flower and seed kernel of Mangifera indica L.* Int. J. Pharm. Bio. Sci, 2013. **4**(2): p. 383-389.
15. Banu, K.S. and L. Cathrine, *General techniques involved in phytochemical analysis.* International journal of advanced research in chemical science, 2015. **2**(4): p. 25-32.
16. Edeoga, H.O., D. Okwu, and B. Mbaebie, *Phytochemical constituents of some Nigerian medicinal plants.* African journal of biotechnology, 2005. **4**(7): p. 685-688.
17. Krishnaveni, G., O. Sailaja, and K.K. Kumar, *Phytochemical screening and quantitative analysis of hexane, acetone, methanol and water extracts of Salicornia virginica by UV-spectrophotometry.* Der Pharmacia Lettre, 2016. **8**(16): p. 52-56.
18. Devmurari, V., *Phytochemical screening study and antibacterial evaluation of Symplocos racemosa Roxb.* Archives of applied science research, 2010. **2**(1): p. 354-359.
19. Saini, S., A. Dhiman, and S. Nanda, *Pharmacognostical and phytochemical studies of Piper betle Linn. leaf.* Int J Pharm Pharm Sci, 2016. **8**(5): p. 222-226.
20. Chitravadivu, C., S. Manian, and K. Kalaichelvi, *Qualitative analysis of selected medicinal plants, Tamilnadu, India.* Middle East Journal of Scientific Research, 2009. **4**(3): p. 144-146.

21. Harborne, A., *Phytochemical methods a guide to modern techniques of plant analysis*. 1998: springer science & business media.
22. Ali, S., et al., *Phytochemical investigation and antimicrobial appraisal of Parrotiopsis jacquemontiana (Decne) Rehder.* BMC complementary and alternative medicine, 2018. **18**: p. 1-15.
23. Snehlata, K., R. Sheel, and B. Kumar, *Evaluation of phytochemicals in polar and non polar solvent extracts of leaves of Aegle marmelos (L.).* Journal of Biotechnology and Biochemistry, 2018. **4**(5): p. 31-38.
24. Dahiru, D., J. Onubiyi, and H.A. Umaru, *Phytochemical screening and antiulcerogenic effect of Moringa oleifera aqueous leaf extract.* African Journal of Traditional, Complementary and Alternative Medicines, 2006. **3**(3): p. 70-75.