**Phytochemical screening of *Calligonum polygonoides* stem extract collected from Jaisalmer district of Rajasthan, India**

**ABSTRACT**

This study aimed to qualitatively analyze the hydroalcoholic stem extracts of *Calligonum polygonoides* to identify major secondary metabolites with potential pharmacological significance*. C. polygonoides,* commonly known as “Phog” a plant native to the arid regions of Rajasthan, India, thrives under extreme desert conditions characterized by high temperatures, intense solar radiation, and limited water availability. This study aimed to qualitatively analyze the hydroalcoholic stem extracts of *C. polygonoides* to identify major secondary metabolites with potential pharmacological significance. Plant samples were collected from Chelak Village, Jaisalmer, a region within the Thar Desert, where plants have evolved unique biochemical adaptations to survive harsh environmental stresses. The stem of *C. polygonoides* was collected, dried, and ground into a fine powder, followed by hydroalcoholic extraction. Standard phytochemical tests were conducted to detect key bioactive compounds. The qualitative screening confirmed the presence of flavonoids, tannins, phenolic compounds, saponins, sterols, quinones, glycosides, and alkaloids in the stem extracts. These secondary metabolites are renowned for their diverse therapeutic properties, comprising anti-oxidant, anti-inflammatory, antimicrobial, and anticancer properties. The occurrence of these bioactive compounds in *C. polygonoides* underscores its potential as a valuable source of plant-derived pharmaceuticals. The ability of this plant to produce a rich array of phytochemicals despite extreme desert conditions suggests its evolutionary adaptation to environmental stress, which may contribute to its medicinal properties. This study highlights the significance of exploring desert flora for novel bioactive compounds that could lead to the development of sustainable, plant-based therapeutic agents.

**Keywords:** *Calligonum polygonoides;* Jaisalmer; Phog; Phytochemicals.

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1. **INTRODUCTION**

 The herbal medicinal system has been developed and refined over time through empirical observations and exploratory experiments, serving as a means to promote health and treat various ailments and diseases since ancient times (Karunamoorthi et al., 2013). Plants have long been employed as traditional herbal remedies for a variety of illnesses, and the numerous natural products they produce have served as inspiration for the creation of novel medications (Chaachouay and Zidane, 2024). In recent years, there has been increased interest in the study of medicinal plants, which are rich in phytochemicals beneficial to human health. Since ancient times, various plant-derived secondary metabolites have been utilized as key components in drug formulations to treat numerous human disorders. The traditional therapeutic uses of these secondary metabolites have been documented worldwide. Advanced techniques have enabled the identification and isolation of numerous bioactive phytochemicals, which are responsible for a wide array of pharmacological properties, including anticancer, anti-inflammatory, anti-allergic, and antimicrobial effects (Kaushik et al., 2021). Phytochemicals are naturally occurring bioactive compounds available in fruits, nuts, grains, vegetables, tea and seeds, playing a crucial role in promoting health and prevention of diseases. Therapeutic properties of medicinal plants are largely because of their rich phytochemical content, particularly flavonoids, terpenoids, alkaloids, phenolic acids, lignans, sterols, tannins, stilbenes, and saponins (Nyamai et al., 2016). The antioxidant and medicinal potential of many secondary metabolites, including as phenolics, terpenes, and alkaloids, has been studied. Numerous investigations examined the particular compounds with important therapeutic qualities (antioxidant, anti-inflammatory, anti-cancer, and antibacterial), their modes of action, and possible uses in pharmacology and medicine (Hilal et al., 2024).

 Medicinal plants are a vital source for traditional medicine, contemporary pharmaceuticals, nutraceuticals, dietary supplements, folk remedies, pharmaceutical precursors, and chemical intermediates for synthetic drug development. Their therapeutic effects are largely attributed to the diverse secondary metabolites they contain. The medicinal properties of specific plant species or groups are distinct, as the unique combination of secondary metabolites within each plant is often taxonomically specific (Saranraj et al., 2016). Recent studies have highlighted the importance of phytochemical screening in evaluating the therapeutic potential of plants. Several plants have been explored for their biological activities based on the phytochemicals specially secondary metabolites present within such as Neem (*Azadirachta indica*) contain a diverse range of bioactive compounds that can be extracted and utilized in herbal medicine (Patel et al., 2024). *Chlorophytum borivilianum* exhibits anti-tumour, anti-mutagenic, and chemomodulatory effects(Kumar et al., 2010). *Martynia annua* exhibited various pharmacological activities, including antibacterial, anthelmintic, antioxidant, and wound-healing activities (Sharma et al., 2024). *Evolvulus parviflora* identified bioactive compounds with significant antimicrobial and antioxidant properties (Adil et al., 2024). These findings highlight the critical role of phytochemical screening in discovering plant-based therapeutic agents.

 Identifying phytoconstituents in plant material helps predict its potential pharmacological activity. Phytochemical analysis is essential for evaluating a plant's potential medicinal uses and identifying the active compounds responsible for its biological activities. Additionally, it serves as a foundation for targeted compound isolation and more precise investigations (Shaikh and Patil, 2020). Phytochemicals have an important role in combating several diseases, including arthritis, asthma, and cancer. Unlike pharmaceutical chemicals, they no or very less adverse effects. Since they treat diseases without harming humans, they can be regarded as " biocompatible therapeutics" (Banu and Cathrine, 2015). *Calligonum polygonoides* commonly known as “Phog” is recognized as a keystone species in the Thar Desert ecosystem (Vyas et al., 2012).  It is naturally found in North African, Southern European, Central and Western Asian Deserts (Swarnkar et al., 2019). The consideration of *C. polygonoides* in pharmaceutical research holds great promise, as detailed studies may identify novel bioactive secondary metabolites with therapeutic potential to address various health issues (Meghwal et al., 2024).

 This study aims to conduct a preliminary qualitative phytochemical screening of *C. polygonoides* extracts to identify key secondary metabolites. The findings will enhance the understanding of plant-based bioactive compounds and may guide future research on the development of plant-derived therapeutic agents.

1. **MATERIALS AND METHODS**
	1. **Plant Material Collection and Extract Preparation**

 *C. polygonoides* stems were collected in winter from Chelak Village in Jaisalmer District, Rajasthan, India (26.508917°N, 70.908462°E). They were verified using a voucher specimen (RUBL-211762) that was kept in the Department of Botany's herbarium at the University of Rajasthan, Jaipur. To optimize the quantity of bioactive components, a hydroalcoholic extract of the stems was made using Soxhlet extraction with a 3:1 methanol-water solvent mixture. The solvent was evaporated to concentrate the *C. polygonoides* stems extract (CPSE), which was then dried and kept at room temperature in an airtight container.

* 1. **Qualitative Phytochemical Screening**

 Various standard methods were used to qualitatively detect the presence or absence of specific secondary metabolites in the stem extract of *C. polygonoides*.

* + 1. ***Flavonoid*:**

 Shinoda’s test is a qualitative assay used to identify flavonoids in plant extracts by reducing them with magnesium (Mg) in the presence of concentrated hydrochloric acid (HCl). Plant extract (1–2 mL) was combined with a small piece of magnesium ribbon or powder, followed by the addition of a 2-3 drops of HCl. A positive result was denoted by the development of a pink, red, or orange colour, confirming the presence of flavonoids. Quercetin was used as standard. This colour change occurs due to the reduction of flavonoid compounds, particularly flavones, flavonols, and flavanones, making Shinoda’s test a simple and reliable method for preliminary flavonoid screening in plant-based research (Nanna et al., 2013).

* + 1. ***Saponin*:**

 The foam test (Kashyp et al., 2023) is a simple qualitative method used to detect saponins in plant extracts. In this test, a small amount of the sample (0.5mg extract) was mixed with water (5ml) and shaken vigorously. If saponins were present, the mixture forms a persistent foam that lasts for several minutes due to the surfactant properties of saponins, which have both hydrophilic and hydrophobic components that reduce surface tension. This test is commonly used for the preliminary identification of saponins in plant-based samples, as their unique ability to stabilize air bubbles in water results in the formation of a stable foam. Diosgenin was used as a positive control for saponin detection.

* + 1. ***Quinones*:**

 The sulfuric acid test is a simple qualitative method used to detect quinones in plant extracts. Concentrated sulfuric acid (2-3 drops) were added to the sample (10mg extract dissolved in isopropyl alcohol), and the formation of a red, yellow, or orange coloration or a yellow or red ring at the interface indicated the presence of quinones. This colour change occurs due to the reaction of quinones with sulfuric acid, which acts as a dehydrating agent, facilitating the formation of a coloured complex. For quinone detection, 1,4-Benzoquinone was used as standard. The test is widely used for the preliminary screening of quinones, which are known for their characteristic aromatic structure and conjugated systems (Maria et al., 2017).

* + 1. ***Phenols*:**

 The Ferric Chloride Test, is a qualitative assay employed to detect phenolic compounds in samples. In this method, 2-3 drops of 5% ferric chloride solution were mixed to the sample, resulting a red, blue, green, or purple coloration if phenols were present. This colour change occurs due to the formation of ferric-phenolate complexes. For phenols detection, gallic acid was used as a positive control. The test is valued for its simplicity and effectiveness in preliminary phenolic screening in various studies (Santhi and Sengottuvel, 2016).

* + 1. ***Glycosides*:**

 The Keller-Killani test is a qualitative method used to detect the presence of cardiac glycosides in plant extracts. In this assay, 1.5mL glacial acetic acid, 2 drops of 5% ferric chloride solution, and concentrated sulfuric acid were added to the sample. The appearance of a red or reddish-brown colour at the interface or a colour change from green to red indicated the presence of glycosides, specifically cardiac glycosides. Digitoxin was used as standard for detecting of glycosides. The test works due to the interaction between the glycoside and iron ions from ferric chloride, with sulfuric acid enhancing the colour reaction (Singh and Kumar, 2017).

* + 1. ***Tannin*:**

 The Ferric Chloride Test, also known as Braymer’s Test, is a simple qualitative method used to detect tannins in plant extracts. In this test, about 2 mL of the stem extract was mixed with a 10% alcoholic ferric chloride solution. The appearance of a blue or green colour suggest the presence of tannins. This colour change occurs due to the formation of a ferric-phenolate complex, as tannins are phenolic compounds that interact with ferric ions to produce the characteristic coloration. For tannin detection, gallic acid was used as a standard. This test is commonly used for preliminary screening of tannins in plant-based studies and is valued for its simplicity and effectiveness (Uma et al., 2017).

* + 1. ***Sterols*:**

 Hesse’s test is a qualitative technique for identifying sterols in plant extracts and other biological samples. In this method, the stem extract was combined with 2 mL of chloroform, followed by an equal volume of sulfuric acid. The formation of a pink-hued ring, which disperses into both layers upon shaking, indicates the presence of sterols. For screening of sterols, stigmasterol was used as positive control. This test is widely used for its simplicity in detecting sterolic compounds such as cholesterol and phytosterols in plant- and animal-derived samples. (Kumar and Jat, 2018).

* + 1. ***Alkaloid*:**

 Dragendorff’s test is a qualitative method used to detect alkaloids in plant extracts. In this assay, 2-3 drops of Dragendorff’s reagent, which contains bismuth chloride and potassium iodide, were added to the sample. The presence of alkaloids was indicated by the appearance of an orange-brown precipitate, caused by the reaction between alkaloids and the reagent. This occurs because alkaloids, which contain nitrogen, interact with the bismuth ions in the reagent, leading to the formation of the characteristic precipitate. Atropine was used as standard for this test. This test is commonly used for the preliminary identification of alkaloids in plant-based materials (Silva et al., 2017).

1. **RESULTS AND DISCUSSION**

 Qualitative phytochemical analysis of the *C. polygonoides* stem extract revealed the presence of various bioactive compounds. The presence of flavonoids was confirmed by Shinoda’s test, where a pink colour appeared after adding HCl and magnesium powder. Tannins were detected using the ferric chloride test, which produced a blue-black coloration, while phenolic compounds were also identified through the same test, resulting in a blue colour. The foam test indicated the presence of saponins, as persistent foam formed after shaking the sample with water. Hesse’s test confirmed the presence of sterols, producing a red coloration upon adding concentrated sulfuric acid. Quinones were detected through the sulfuric acid test, which developed a yellowish-red colour. Glycosides were identified using the Keller-Killani test, where a reddish-brown ring formed at the interface after adding sulfuric acid and glacial acetic acid. Finally, alkaloids were confirmed through Dragendorff’s test, which yielded an orange-brown precipitate (Table-1).

**Table-1: Phytochemicals present in stem extract of *Calligonum polygonoides.***

|  |  |  |  |
| --- | --- | --- | --- |
| **S.N.** | **Phytochemical** | **Test Results** | **Method Use** |
| 1. | Flavonoid | Present | Shinoda’s test |
| 2. | Saponin | Present | Foam test |
| 3. | Quinones | Present | Sulphuric acid test |
| 4. | Phenol | Present | Ferric chloride test |
| 5. | Glycosides | Present | Keller-Killani test |
| 6. | Tannin  | Present | Ferric chloride test |
| 7. | Sterols | Present | Hesse's test |
| 8. | Alkaloid | Present | Dragendroff’s test |

The qualitative phytochemical analysis of *C. polygonoides* extract confirmed the presence of several bioactive compounds, including flavonoids, phenolics, tannins, saponins, sterols, quinones, glycosides, and alkaloids. These secondary metabolites play a critical role in the pharmacological properties of medicinal plants. The detection of flavonoids through Shinoda’s test indicates the potential antioxidant activity of *C. polygonoides*, as flavonoids are known to scavenge free radicals and mitigate oxidative stress (Kumar and Pandey, 2013). Similarly, the presence of phenolics and tannins, confirmed by the Folin-Ciocalteu method and Ferric chloride test, suggests the plant's potential antimicrobial and anti-inflammatory effects, as these compounds exhibit strong antibacterial properties (Cheynier, 2012). A positive result in the Vanillin-sulfuric acid test suggests that *C. polygonoides* contains bioactive saponins, which have been linked to immunomodulatory and cytotoxic effects (Sparg et al., 2004). The presence of sterols, confirmed by Hesse’s test, may contribute to anti-inflammatory and cholesterol-lowering activities (Yalcinkaya et al., 2024). Additionally, the Sulfuric acid test indicated the presence of quinones, which possess antimicrobial and anticancer properties (Wang et al., 2016). The detection of cardiac glycosides through the Keller-Killani test supports their cardioprotective potential (Maitra and Nath, 2019). Overall, the presence of these phytochemicals in *C. polygonoides* reinforces its traditional medicinal applications and underscores its potential as a valuable source of bioactive compounds for pharmaceutical development.

1. **CONCLUSION**

 The qualitative phytochemical analysis of Calligonum polygonoides confirmed the presence of flavonoids, phenolics, tannins, saponins, sterols, quinones, glycosides, and alkaloids. These bioactive compounds exhibit antioxidant, anti-inflammatory, and antimicrobial properties, highlighting the plant’s potential for novel pharmaceutical applications. These findings highlight the plant’s potential for novel bioactive compounds in drug discovery. Future research should explore its therapeutic mechanisms, bioavailability, and clinical efficacy for drug development.

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**AUTHORS’ CONTRIBUTIONS**

Priyadarshi Meena and Dev Dutt Patel conceptualized, designed the study and supervised the analysis. Gyan Prakash Meghwal collected the sample material and drafted the experimental protocol. Gyan Prakash Meghwal, Mahendra Kumar Jeengar, and Kamlesh Kumar Sharma conducted the analyses and prepared the initial manuscript draft. All authors reviewed and approved the final manuscript.

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**REFERENCES**

Adil M, Filimban FZ, Ambrin, Quddoos A, Sher AA & Naseer M. (2024). Phytochemical screening, HPLC analysis, antimicrobial and antioxidant effect of *Euphorbia parviflora* L. (Euphorbiaceae Juss.). *Scientific reports*, *14*(1), 5627.

Banu KS & Cathrine L. (2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, *2*(4), 25-32.

Chaachouay, N., & Zidane, L. (2024). Plant-derived natural products: a source for drug discovery and development. *Drugs and Drug Candidates*, *3*(1), 184-207.

Cheynier V. (2012). Phenolic compounds: From plants to food and health. *Phytochemistry Reviews, 11*(2-3), 153-177.

Hilal, B., Khan, M. M., & Fariduddin, Q. (2024). Recent advancements in deciphering the therapeutic properties of plant secondary metabolites: phenolics, terpenes, and alkaloids. *Plant physiology and biochemistry: PPB*, 211, 108674.

Karunamoorthi K, Jegajeevanram K, Vijayalakshmi J & Mengistie E. (2013). Traditional medicinal plants: a source of phytotherapeutic modality in resource-constrained health care settings. *Journal of Evidence-Based Complementary & Alternative Medicine*, 18(1), 67-74.

Kashyp, K., Das, A. K., Bhardwaj, A. K., Roymahapatra, G., Ghosh, A., Hiat, M., & Jain, R. (2023). Phytochemical analysis of Careya arborea Roxb. root extracts: a qualitative analytical approach. *ES General*, *1*(4), 959.

Kaushik B, Sharma J, Kumar P & Shourie A. (2021). Phytochemical properties and pharmacological role of plants: secondary metabolites. *Biosciences Biotechnology Research Asia*, *18*(1), 23.

Kumar M, Meena P, Verma S, Kumar M & Kumar A. (2010). Anti-tumour, Anti-mutagenic and Chemomodulatory Potential of *Chlorophytum borivilianum. Asian Pacific J Cancer Prev.*; 11: 327-334.

Kumar S & Pandey AK. (2013). Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal, 2013*, 162750.

Kumar V & Jat RK. (2018). Phytochemical estimation of medicinal plant achyranthes aspera root. *International Journal of Research in Pharmacy and Pharmaceutical Sciences.* 3(1), 190-193.

Maitra S & Nath A. (2019). Cardiac glycosides: From plants to clinical use. *Pharmacognosy Reviews, 13*(25), 62-68.

Maria R, Shirley M, Xavier C, Jaime S, David V, Rosa S & Jodie D. (2017). Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. *Journal of King Saud University - Science*, 30(4), 500–505.

Meghwal GP, Jeengar MK, Patel DD, Meena P. Phog: A nutraceutical shrub of Thar desert. *Journal of Pharmacognosy and Phytochemistry*; 2024; 13(4): 161-166.

Nanna RS, Banala M, Pamulaparthi A, Kurra A & Kagithoju S. (2013). Evaluation of Phytochemicals and Fluorescent Analysis of Seed and Leaf Extracts of Cajanus cajan L. *International Journal of Pharmaceutical Sciences Review and Research*. 22(1):11-18.

Nyamai DW, Arika W, Ogola PE, Njagi EN & Ngugi MP. (2016). Medicinally important phytochemicals: an untapped research avenue. *Journal of pharmacognosy and phytochemistry*, *4*(4), 2321-6182.

Patel DD, Bilkhiwal N, Parashar R, Jangeer S, Sharma G, Meena P, Meena MK & Yadav RK. (2024). Azadirachta: Biodiversity and its Impact on Health. *Indian J. Applied & Pure Bio. Vol*, *39*(2), 1040-1056.

Santhi KS & Sengottuvel R. (2016). Qualitative and Quantitative Phytochemical analysis of Moringa concanensis Nimmo. *International Journal of Current Microbiology and Applied Sciences*. 5. 633-640.

Saranraj P, Sivasakthi S & Deepa MS. (2016). Phytochemistry of pharmacologically important medicinal plants– A Review. *Int. J. Curr. Res. Chem. Pharm. Sci*., 3(11): 56-66.

Shaikh JR & Patil M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International journal of chemical studies*, *8*(2), 603-608.

Sharma KK, Jeengar MK, Patel DD and Meena P (2024) Baghnakh (*Martynia annua*): Prehistory to contemporary medicinal value. *Journal of Pharmacognosy and Phytochemistry* 13(6): 409-415

Silva GO, Abeysundara AT & Aponso MM. (2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *American Journal of Essential Oils and Natural Products*. 5(2):29-32.

Singh V & Kumar R. (2017). Study of Phytochemical Analysis and Antioxidant Activity of Allium sativum of Bundelkhand Region. *International Journal of Life Sciences Scientific Research*. 3(6):1451-1458.

Sparg SG, Light ME & Staden J. (2004). Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology, 94*(2-3), 219-243.

Swarnkar SK, Khunteta A, Gupta MK, Jain P & Paliwal S. (2019). Pharmacognostic, phytochemical and pharmacological review of “Phog”-*Calligonum polygonoides* L. *J Drug Deliv Ther*, 9(2):469–73.

Uma KS, Parthiban P & Kalpana S. (2017). Pharmacognostical and preliminary phytochemical screening of Aavaarai Vidhai Chooranam. *Asian Journal of Pharmaceutical and Clinical Research*. 10, 111-116.

Vyas GK, Kumar V, Sharma R, Sharma RA, Sharma S, Singh JP & Kumar S. (2012) Chemical and genetic diversity among some wild stands of *Calligonum polygonoides* (Polygonaceae) from the Thar Desert of Rajasthan. *Rev Biol Trop*.; 60:1097–108.

Wang Y, Zhang J, Zhang D & Li H. (2016). Quinones and their anti-cancer activity. *Medicinal Chemistry Research, 25*(5), 875-885.

Yalcinkaya, A., Oztas, Y. E., & Sabuncuoglu, S. (2024). Sterols in Inflammatory Diseases: Implications and Clinical Utility. *Advances in experimental medicine and biology*, *1440*, 261–275.