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Research Article

Qualitative and quantitative determination of phytochemicals in *Convolvulus leiocalycinus* and *Haloxylon griffithii*

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Abstract

The purpose of the present assessment was to evaluate the phytochemicals in the indigenous plants *Convolvulus leiocalycinus* of Convolvulaceae family and *Haloxylon griffithii* of Chenopodiaceae family found in the northern regions of Balochistan. For the analysis of phytochemicals, both plants showed the presence of alkaloids, flavonoids, terpenoids, tannins, saponins, carbohydrates, cardiac glycosides, reducing sugars, quinones and carboxylic acids. Whereas, both plants exhibited negative tests for terpenoids, anthraquinones, phlobatannins, fats, xanthoprotien, resins, anthocyanins, emodins and volatile oil. Furthermore, with the help of standard chemical tests and techniques, the quantitative analysis of *C. leiocalycinus* and *H. griffithii* showed different amounts and percentages of total alkaloids, flavonoids, saponins and phenolics. The total alkaloid contents determined were 0.196(W/w) with percentage yield 3.92% and 0.224(W/w) with percentage yield 4.48% in 5gm of each sample of *C. leiocalycinus* and *H. griffithii* respectively. The total flavonoid contents determined were 0.28(W/w) with 2.8% and 0.445(W/w) with 4.45% in 10gm of each sample of both the plants. The total saponin contents determined were 0.72(W/w) with 3.6% and 0.53(W/w) with 2.65% in 20gm of each sample of both the plants. The total phenolic contents determined were 0.808(W/w) with 16.16% and 0.268(W/w) with 5.36% in 5gm of each sample of both the plants. The presence of high amount of phytochemical compounds suggest that both plants i.e., *C. leiocalycinus* and *H. griffithii* are not only useful to human beings but can also be commercialized for higher production of natural drugs rather than using synthetic drugs with side effects.

Keywords: *Convolvulus leiocalycinus*; Extraction; *Haloxylon griffithii*; Phytochemicals; Phytochemical screening

Introduction

Phytochemicals are plants based naturally occurring substances. More than ten thousand of these phytochemicals have been identified and many still unknown **[1]**. Phytochemicals include alkaloids, flavonoids, steroids, terpenoids, tannins, phenols, glycosides etc. **[2]**. The therapeutic use of phytochemicals or medicinal plants have been reported in the

ancient traditions and cultures of several societies for many centuries. The reason for their wide therapeutic use are low cost and safe nature than synthetic molecules [3, 4]. Therapeutic processes of phytochemicals involve many biological and pharmacological effects such as anti-tumoral, anti-microbial, pro-oxidant or anti-oxidant, anti-inflammatory, antiviral and anti-mutagenic activities [5-7]. Nowadays, positive results in clinical trials have attracted many phytochemicals into medical practices [8]. In addition to therapeutic effects, phytochemicals are also used as coloring, flavoring and aromatic agents from time immemorial [9].

As stated by World Health Organization (WHO), around 80% people in developing states depend on traditional drugs for their main health requirements and about 85% of these medicines are the plant extracts [10]. The fertile land of Pakistan has a wide variety of medicinal plants and many of these need to be explored for their chemical constituents. Such information would be helpful in determining the actual value of folkloric remedies [11].

This research study involves for the first time, collection, identification, extraction and phytochemical screening of *C. leiocalycinus* and *H. griffithii*. Furthermore, *C. leiocalycinus* is under shrub having woody branches covered with spines, leaves 12-17 (-24) x (3-) 5-7mm, flowers axillary, solitary, ovary glabrous, style c. 9mm long, c. 3 times the length of the cylindrical stigma. *C. leiocalycinus* belongs to the family Convolvulaceae. They are annual or perennial shrub found in higher mountainous regions and rugged slopes of Balochistan (Pakistan) [12].

On the other hand, *H. griffithii* is a genus of shrubs or small trees, belonging to the plant family Chenopodiacea, floral leaves reduced on short spikes. Flowers quite distant. Perianth segments ovate, obtuse, till 1.5mm tall. stamens 5; having fruiting perianth wings of white to pinkish or yellowish in color. A very common bush in Balochistan & Khyber Pakhtunkhwa provinces of Pakistan with few records from Chitral and Gilgit. It is also distributed in Afghanistan and Central Asia [13].

Materials and methods

Sampling

Different parts of *C. leiocalycinus* and *H. griffithii* such as stems, branches, leaves, flowers and roots were collected from different locations of Hanna Lake, Urak region and Spinni road of Quetta, Balochistan on 3rd of September 2017. The samples were identified by Rasool Bakhsh Tareen, an eminent Botanist, University of Balochistan, Quetta. The samples were dried in shadow, grounded into powder form and sieved (Mesh No 325). The powdered samples were stored in plastic zip bags before use.

Extraction of plant material

The powdered plant substance weighed 4kg was soaked in 15-liter methanol (99.8%) for a period of 7 days. The mixture was filtered by Whatman filter paper No.40 (125mm) followed by re-extraction of the remaining crude with methanol (99.8%) 5 liters unless the color of the solvent changed. Methanol was removed using a rotary evaporator and the dry crude methanolic extract (CME) of *C. leiocalycinus* having weight 200g and *H. griffithii* having weight 210g were extracted. The CME of both plants were stored in an airtight container in a cool, dark and dry place and were used for further phytochemical screening.

Phytochemical screening

The plant extracts were further used for the analysis of various phytochemicals qualitatively and quantitatively.

Qualitative phytochemical analysis

The qualitative analysis of phytochemicals such as alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids, carbohydrates, glycosides, quinones, anthraquinones, phlobatannins, fats, xanthoprotein, resins, carboxylic acids, anthocyanins, emodins and volatile oil were carried out using several standard analytical techniques and procedures.

Test for alkaloids

For alkaloids, 0.55–0.65g of the crude methanolic extract was mixed with 8.5ml of 1% hydrochloric acid followed by warming and filtration. The filtrate was divided into three equal parts in separate test tubes and blended with Wagner's chemical, Mayer's chemical and Dragandroff's reagent and then noted the precipitate formation and color change **[14].**

Test for flavonoids

For testing the presence of flavonoids, the plant extract was first treated with petroleum ether for the removal of fats. To the remnant, 80% of 20.5ml ethanol was blended and strained. The remainder was utilized for the tests mentioned below.

1. The remainder was treated with 4.5ml of 1% potassium hydroxide and examined the color change **[15]**.
2. The presence of flavonoids was also checked by treating the filtrate with 5.5ml of ammonia solution and condensed sulphuric acid and examined the color alteration **[16]**.

Test for tannins

0.55g of plant extract was liquified in 20.5ml of purified water and was strained. The remainder was treated with 0.1% of ferric chloride and a brownish green color was noted for the presence of tannins **[17]**.

Test for phenolics

Two procedures were followed to test the presence of phenolics. They were:

1. The plant CME was blended with a few drops of neutral ferric chloride solution and intense violet color change was examined.
2. For another test of phenolics, a little amount of each plant extract was first dissolved in purified water and then (3.5ml) of 3% lead acetate was mixed with plant extract, after which formation of bulky white precipitates was observed **[18]**.

Tests for saponins

For saponins, 0.55g of plant essence was quivered with distilled water and was warmed until it boiled. The blend was shuddered to lather emergence **[19]**.

Test for carbohydrates

1. Molisch's test

The plant extract was treated with 10% alcoholic alpha naphthol followed by the addition of 2.5ml sulphuric acid. The presence of carbohydrates and glycosides were confirmed by the appearance of bluish violet region.

2. Fehling's test

To perform Fehling's test for carbohydrate's presence, the Fehling's solution was prepared and was labeled as A and B. From both the solutions, 5.5ml was added to the plant essence and was heated. The development of reddish brown precipitates was observed for the presence of reducing sugars.

Detection of cardiac glycosides

For cardiac glycosides, 2.5ml of glacial acetic acid was treated with 0.55ml of the plant essence, with the addition of few drops of ferric chloride (5%). To this blend, 1ml of sulphuric acid was mixed and development of a brown ring showed the presence of cardiac glycosides **[20]**.

Test for quinones

1.5ml of sulphuric acid was treated with plant extract and a red color formation was noted showing the presence of quinones **[21]**.

Carboxylic acids

For carboxylic acids, 3ml methanolic extract was mixed with 3ml of sodium bicarbonate solution. Effervescence due to carbon dioxide showed the presence of carboxylic acids **[22]**.

Quantitative phytochemical analysis

Quantitative analysis of the plants extract was conducted to evaluate the amounts and percentages of various phytochemicals such as total alkaloids, flavonoids, phenolics and saponins by means of standard chemical tests and techniques.

Test for total alkaloids

Quantitative estimation of alkaloid was performed by using the procedure of Harborne. Exactly 200cm^3 of 10% acetic acid in ethanol was mixed with each plant powder sample 5gm in a 250cm^3 beaker and kept for 4 hours. The essence was condensed on a water bath to one-fourth of the actual magnitude and then 15 drops of

condensed ammonium hydroxide were added drop by drop to the extract. The floating substance was removed, and the precipitates were cleaned through 20cm^3 of 0.1 M ammonium hydroxide solution and then sieved. The filtrate was desiccated in an oven and weighed by electronic balance and the percentage of alkaloid was calculated [23].

Test for flavonoids

Flavonoids were determined by the procedure described by Bohm and Kocipai-Abyazan. Precisely, 50cm^3 of 80% aqueous methanol was blended to 10gm of sample in a 250cm^3 beaker enveloped and kept for 24 hours at normal temperature. After disposal the floating substance, the remainder was extracted thrice with the identical magnitude of ethanol once more. Whatman filter paper having number 42 (125 mm) was utilized to sieve the entire solution of wood specimen. Each specimen remainder was later shifted into a container and desiccated above a water bath. The material in the crucible was cooled through desiccator, weighed and calculated the percentage of flavonoids [24].

Test for saponins

Saponin quantitative estimation was performed by means of the modern procedure. Accurately, 100cm^3 of 20% aqueous ethanol was mixed with 20 grams of each plant powder sample in a 250cm^3 conical flask. The mixture was heated above a hot water bath for 4 hours with constant stirring at a temperature of almost 55°C. The remainder of the mixture was extracted once more with another 100cm^3 of 20% aqueous ethanol after filtration and heated for 4 hours at a persistent temperature of 55°C with continuous stirring. The collective decoction was vaporized to 40cm^3 above water bath at 90°C. After this, 20cm^3 of diethyl ether was mixed to the distillate in a 250cm^3 separating funnel and strongly blended. Afterward, the aqueous layer was retrieved whereas the ether layer was removed. This refinement procedure was repeated two times. Then, 60cm^3 of n-butanol was mixed and extracted two times with 10cm^3 of 5% sodium chloride. Later removing the sodium chloride layer, the residual solution was heated through water bath for half an hour. After that, the solution was shifted into a container and was desiccated by an oven to get a constant weight. The saponin extract was weighed and computed the percentage [25].

Test for total phenolics

The estimation of total phenolics was accomplished quantitatively using the procedure described by Hagerman. Through this procedure, 5g of powder plant samples were treated separately with 200ml of n-hexane twice for 3 hours each. The filtrate was removed from the residue for the preparation of fat free sample. Then residue was heated on water bath for 20 minutes with 100ml diethyl ether twice and then cooled to room temperature. The solution was filtered and was transferred in a separating funnel. The filtrate was treated with 50ml of 10% NaOH solution two times and was quivered well each time. The organic layer was isolated from the aqueous layer. It was washed two times with 50ml deionized water. The aqueous layer was acidulated up to pH 4 by the addition of 10% HCl solution and 100ml dichloromethane. Subsequently, the organic layer was collected and dried over water bath, weighed and determined the percentage of phenolics [26].

Results and discussion

The purpose of the current investigation is to assess the phytochemical composition of the methanolic extract of *C. leiocalycinus* of Convolvulaceae family and *H. griffithii* of the Chenopodiaceae family by qualitative methods and also to determine the amounts of phytochemicals by quantitative analysis using various standard procedures.

Natural products are not only a good source of phytochemicals utilized as drugs but also assist in the manufacture of new effective synthetic drugs. The phytochemical analysis of these indigenous plants showed very exciting results. The screening of the

phytochemicals extracted from these plants showed positive tests for the presence of alkaloids, flavonoids, tannins, phenolics, saponins, carbohydrates, glycosides, quinones and carboxylic acids given in the (Table 1). However, phytochemicals such as terpenoids, anthraquinones, phlobatannins, fats, xanthoprotien, resins, emodins, volatile oil, and anthocyanins showed negative tests for both plants given in the (Table 2).

Quantitative Analysis

The quantitative estimation of the examined plants revealed that phytochemicals are present in different amounts in each plant extract. The total alkaloid contents determined were 0.196(W/w) with percentage yield 3.92% and 0.224(W/w) with percentage yield 4.48% in 5gm of each sample of the *C. leiocalycinus* and *H. griffithii*. The result showed that alkaloid content was greater in *H. griffithii* than *C. leiocalycinus* (Table 3). Similarly, the total flavonoid contents determined were 0.28(W/w) with percentage yield 2.8% and 0.445(W/w) with percentage yield 4.45% in 10gm of each sample of both the plants. The flavonoid content was higher in *H. griffithii* than *C. leiocalycinus* (Table 4).

Moreover, the total saponin contents determined were 0.72(W/w) with percentage yield 3.6% and 0.53(W/w) with percentage yield 2.65% in 20gm of each sample of both the plants. The results revealed that the concentration of saponins was higher in *C. leiocalycinus* as compared to *H. griffithii* (Table 5).

Consequently, the total phenolic contents determined were 0.808(W/w) with percentage yield 16.16% and 0.268(W/w) with percentage yield 5.36% in 5gm of each sample of *C. leiocalycinus* and *H. griffithii*. The phenolic contents were higher in *C. leiocalycinus* than *H. griffithii* (Table 6). Concluding these results, it can be simply judged that the phenolic contents are higher than the contents of alkaloids, flavonoids and saponins of both the plants.

The results from the quantitative analysis showed significant variations among the contents of alkaloids, flavonoids, saponins and phenolics when compared with one other. These variations are due to number of environmental factors such as climate, altitude, rainfall etc. **[27]**.

From the literature survey it was found that alkaloids and phenolic compounds show antidiabetic properties, anti-inflammatory, antimicrobial and antioxidant effects. Similarly, flavonoids have wide range of biological properties such as anti-inflammatory, antibacterial, antiviral, anti-allergic, cytotoxic and antitumor properties. It is used in the treatment of neurodegenerative diseases and has vasodilatory action.

In addition, saponins exhibit antimicrobial activity extremely to coldblooded animals, but toxicity to mammals is low. Saponins are mild detergent utilized in intracellular histochemistry staining to allow antibody access to intracellular proteins. The saponins are used in hypercholestrolaemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory activity and weight loss **[28]**.

Tannins are also involved in treatment of non-insulin dependent diabetes mellitus by enhancing the glucose uptake and inhibiting adipogenesis. The most striking feature about quinones is their pharmacological properties that make them different from other secondary metabolites. It inhibits HIV 1 reverse transcriptase and shows antitumor and immunomodulatory activities. It also has antimicrobial, anticancer, antiviral and antibacterial properties **[29]**.

Table 1. It shows the summary of the positive tests of different phytochemicals in *C. leiocalycinus* and *H. griffithii*

S. No	Phytochemicals	Tests	Observation	Results	
				Convolvulus leiocalycinus	***Haloxylon griffithii***
1	Alkaloids	2ml of plant essence + few drops of Wagner's reagent	Red precipitates are formed	+	+
	″ ″ ″ ″ ″ ″	1ml filtrate + few drops of Mayer's chemical.	white precipitates appeared	+	+
	″ ″ ″ ″ ″ ″	1ml of filtrate + 2ml of Dragandroff's reagent	Yellow precipitate appeared	+	+
2	Phenols	Plant essence + few drops of neutral $FeCl_3$ solution	Intense color developed	+	+
	″ ″ ″ ″ ″ ″	0.5g plant essence dissolved in distilled H_2O + 3ml of 10% lead acetate	Bulky white precipitates appeared	+	+
3	Saponins	0.5g of essence was dissolved in boiling water in a test tube, was cooled and shaken vigorously	Froth formation was observed	+	+
4	Flavonoids	3ml of fats free filtrate + 4ml of 1% potassium hydroxide	Dark yellow color observed	+	+
	″ ″ ″ ″ ″ ″	5ml of dil. ammonia solution + extract + H_2SO_4	Yellow coloration observed, which decolorized on addition of acid.	+	+
5	Tannins	0.5g extract was boiled in 20ml of distilled water, filtered + 0.1% $FeCl_3$	Brownish green or a blue-black coloration observed	+	+
6	Quinone	Plant extract + 1ml conc. sulphuric acid	Formation of red color was observed	+	+
7	Carbohydrates	Molisch's test Water solution of extract + 10% alcoholic alpha naphthol + 2ml sulphuric acid	Appearance of bluish violet zone	+	+
8	Cardiac glycosides	0.5ml of plant concentrate + 2ml of glacial acetic acid + few drops of 5% ferric chloride + 1ml of conc. sulphuric acid	Formation of brown ring at the interface was observed	+	+
9	Carboxylic acids	3ml of plant extract + 3ml $NaHCO_3$ solution	Effervesces due to CO_2 indicate the presence of carboxylic acids	+	+

Table 2. It shows the summary of the negative tests of different phytochemicals in *C. leiocalycinus* and *H. griffithii*

S. No	Phytochemicals	Tests	Observation	Results	
				Convolvulus leiocalycinus	***Haloxylon griffithii***
10	Terpenoids	5ml of extract of each + 2ml $CHCl_3$ + 3ml of H_2SO_4	A boundary with a reddish-brown color observed	-	-
11	Anthraquinones	2ml methanolic extract + few drops of 2% HCl	Red color precipitates	-	-
12	Phlobatannins	2ml methanolic extract + few drops of 10% ammonia solution	Pink color precipitates	-	-
13	Fats	5ml of 0.5N KOH + 10ml of methanolic extract + few drops phenolphthalein + heat for 2-hours	Soap formation or partial neutralization of alkali	-	-
14	Xanthoprotien	2ml methanolic extract + 0.5ml Conc. HNO_3 + 0.5ml NH_3	Reddish orange precipitates	-	-
15	Resins	2ml methanolic extracts + 5-6 drops acetic anhydride solution + 1ml Conc. H_2SO_4	Resins give coloration ranging from orange to red	-	-
16	Anthocyanins	3ml methanolic extracts + 3ml 2NHCl + few drops NH_3 solution	Pinkish red color	-	-
17	Emodins	3ml methanolic extract + 3ml NH_4OH solution + 3ml benzene	Red color	-	-
18	Volatile oil	4ml methanolic extract + 2ml dilute NaOH + 2ml dil. HCl	Formation of white precipitates	-	-

Table 3. It shows the quantities and percentages of alkaloids in *C. leiocalycinus* and *H. griffithii*

Plant species	Weight of sample	Weight of alkaloid	% age of alkaloid = weight of alkaloid x 100 / weight of sample
C. leiocalycinus	5gm	0.196gm	% age of alkaloid = 3.92%
H. griffithii	5gm	0.224gm	% age of alkaloid = 4.48%

Table 4. It shows the quantities and percentages of flavonoids in *C. leiocalycinus* and *H. griffithii*

Plant species	Weight of sample	Weight of flavonoids	% age of flavonoids = weight of flavonoids x 100 / weight of sample
C. leiocalycinus	10gm	0.28gm	% age of flavonoids = 2.8%
H. griffithii	10gm	0.445gm	% age of flavonoids = 4.45%

Table 5. It shows the quantities and percentages of saponins in *C. leiocalycinus* and *H. griffithii*

Plant species	Weight of sample	Weight of saponins	% age of saponins = weight of saponins x 100 / weight of sample
C. leiocalycinus	20gm	0.72gm	% age of saponins = 3.6%
H. griffithii	20gm	0.53gm	% age of saponins = 2.65%

Table 6. It shows the quantities and percentages of phenolics in *C. leiocalycinus* and *H. griffithii*

Plant species	Weight of sample	Weight of phenolics	% age of phenolics = weight of phenolics x 100 / weight of sample
C. leiocalycinus	5gm	0.808gm	% age of phenolics = 16.16%
H. griffithii	5gm	0.268gm	% age of phenolics = 5.36%

Conclusion

This exploration discloses all the phytochemicals present in the plants like *C. leiocalycinus* and *H. griffithii*. This investigation also reveals the concentration of various phytochemicals present in the CME of both the plants by utilizing well established techniques.

C. leiocalycinus and *H. griffithii* are full of pharmacological and medicinal significance and are rich in phytochemicals that could be considered as responsible for their therapeutic effects, antimicrobial, antifungal and anti-oxidant activities. Furthermore, the plants screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers. Additional investigation is required for the isolation and assessment of the bioactivity like antibacterial and antifungal activities of different sections of the CME of *C. leiocalycinus* and *H. griffithii*.

Authors' contributions

Conceived and designed the experiments: M Ahmad, A Baqi & RB Tareen, Performed the experiments: M Ahmad, Samiullah & A Baqi, Analyzed the data: AU Rehman & Samiullah, Contributed materials/ analysis/ tools: N Khan & AU Rehman, Wrote the paper: M Ahmad, A Baqi & A Manan.

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