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Phytochemical Screening of Ethanol and n-hexane Extracts of the Plant Parts and Callus of *Pulicaria jaubertii*

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Abstract

Pulicaria jaubertii is an aromatic and medicinal plant belonging to the family Asteraceae. Previous studies found that this plant contains various important phytochemicals. However, these studies have not covered the identification of these compounds found in its callus produced by micropropagation. This study screens and compares, for the first time, the phytochemicals found in the ethanol and n-hexane extracts of this plant's parts and callus. Native plant parts were collected and washed. Some of them were cultured on Murashige and Skoog medium to produce callus, and the others were dried. Each sample was then powdered and extracted by ethanol and n-hexane solvents and screened for the presence of some biologically active phytochemicals. Screening tests in the ethanol extract revealed the presence of alkaloids, flavonoids, saponins, reducing sugars, cardiac glycosides, carbonyls, phenols/tannins, phlobatanin, terpenoids, and steroids in the callus and most of the plant parts. n-hexane extracts revealed the presence of fewer phytochemical groups in the callus and plant parts. In conclusion, ethanol and n-hexane extracts of plant parts and callus of *P. jaubertii* contain several bioactive compounds. Interestingly, callus extracts revealed some compounds that are not found in certain plant parts. Further studies, including extraction by other solvents, and the use of more advanced separation techniques are recommended.

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Keywords:
Pulicaria jaubertii, Phytochemicals , Callus, Ethanol extract, n-hexane extract

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1. INTRODUCTION

Pulicaria jaubertii E. Gamal-Eldin, which is also called *Pulicaria orientalis* Jaub. and Spach., is an endemic plant to Yemen and is locally known as "Alkhaoah" or "Anssif." It belongs to the family Asteraceae and is traditionally known as a culinary and medicinal plant for several conditions, including fever, urogenital system disorders, malaria, colds, inflammation, and symptoms associated with microbial infections [1–4].

Although it was reported in the literature that this plant has various therapeutic and biological activities, phytochemical studies on this plant are limited [5], and most of the available studies were carried out on the essential oil (EO) extracted from its aerial parts [4–7]. Many studies performed on other species belonging to the same genus [8–16] have revealed the presence of various biologically active compounds like flavonoids, tannins, triterpenoids, saponins, alkaloids, cardiac glycosides, reducing compounds, phytosterols, coumarins, phenols, glucosinolates, terpenoids, and steroids.

Usually, these compounds are obtained from the naturally grown plants. However, obtaining secondary metabolites from them could be hampered by some problems, such as climate change, the possibility of extinction of the plant generation, the probability of obtaining insufficient amounts of bioactive metabolites, and the inability to produce a highly effective and standardized medicine [17–19].

Despite the fact that the natural growth of *P. jaubertii* in the wild is gradually declining [20], some modern techniques could be applied to improve its production, including micropropagation techniques [21, 22]. These techniques involve the induction of the formation of callus, an undifferentiated mass of cells produced *in vitro* from the inoculated explant. Callus culture techniques are considered a useful tool to produce large amounts of active phytochemicals and to yield a large scale of novel products and several classes of therapeutic phytochemicals under controlled conditions [23].

Identifying and comparing these phytochemicals produced from the mother plant with those produced from the yields of micropropagation techniques, including callus culture, is of great importance to identify the benefit of implementing these techniques to improve the amounts and types of these bioactive compounds that could be used for therapeutic purposes [24–26] .

To the best of our knowledge, there are no previous studies investigating the phytochemical constituents of the plant parts or the callus of *P. jaubertii*. This study aimed to screen and compare the bioactive phytochemical classes found in the ethanol and n-hexane extracts of different plant parts of *P. jaubertii* grown in the wild and those found in the callus produced in Murashige and Skoog (MS) medium (1962) [27].

2. MATERIALS AND METHODS

2.1. Study Design and Area

Plant micropropagation was conducted at the Tissue Culture Laboratory, Department of Biology, Faculty of Science, Ibb University, and the extraction and screening of phytochemicals were performed at the Department of Pharmacy, Faculty of Medical Sciences, University of Sciences and Technology, Ibb branch, Yemen, during the year 2021.

2.2. Plant Material

Different parts (leaves, stems, roots, and flowers) of *P. jaubertii* (Figure 1) were collected from different places in Ibb city, Yemen, during the period from March to April 2021.

The sample was identified at the Biology Department, Faculty of Sciences, Ibb University. Samples from each plant part were selected and washed. Some of the collected samples underwent *in vitro* propagation, and the others were dried, powdered, and stored apart in labeled dark glass bottles at the Herbarium of the Faculty of Sciences, Ibb University.

2.3. Methods

2.3.1. Preparation of the Crude Plant for Extraction

Each collected plant part was initially washed thoroughly (3-4 times) with tap water. This was followed by washing with double-distilled water, and then they were dried using blotting papers. The washed plant materials were covered with gauze and kept to dry under ambient shaded conditions for 7 days.

2.3.2. Callus Production

As performed by Salam *et al.,* [28] , the plant parts (explants) were surface-sterilized and inoculated in full MS medium, containing various concentrations and types of cytokinins: Kinetin (Kin) or 6-benzylamino purine (BAP), and auxins: 1-naphthalene acetic acid (NAA) or indole-3-acetic acid (IAA). Seeds were the only explants capable of producing callus successfully within a period of 1-2 weeks. The hormonal formula that yielded the highest callus induction rate and favorable properties was 0.25 mg/L Kin and 1.0 mg/L IAA. All calli obtained from these formulations (Figure 2) were collected after 8 weeks of initial culturing. They were then washed and dried in an oven at 45℃ for a duration of 7 days.

Figure 1. Morphology of *Pulicaria jaubertii* native plant.

The dried plant parts and callus were ground apart to get fine powders using a mixer grinder (GEREMI, China) and were kept at 4˚C in labeled dark glass bottles. Each one of the powders was extracted in the Soxhlet appa-

ratus using ethanol or n-hexane solvent, as performed by Venkatesan *et al.* [29]. Briefly, 100 g of each sample was mixed with 500 mL of the solvent and subjected to hot extraction in the Soxhlet apparatus for 72 hours at a proper temperature below the boiling point of each solvent.

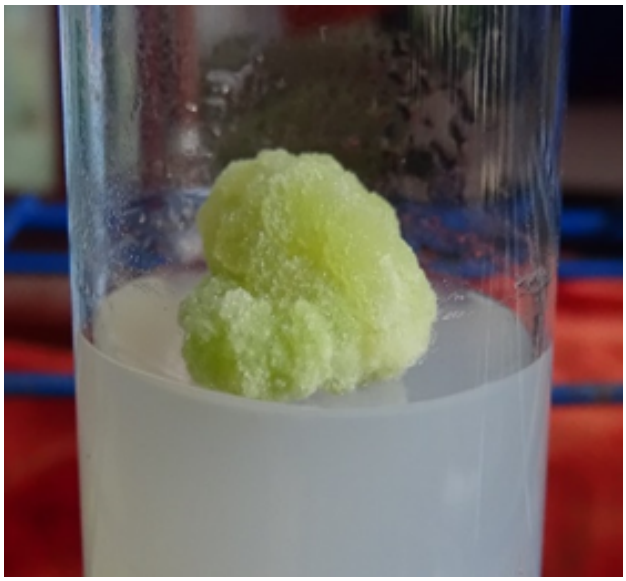

Figure 2. Morphology of *Pulicaria jaubertii* callus produced on MS medium.

2.3.3. Preparation of the Plant Parts and Callus Extracts

The extracts were then filtered by using Whatman filter paper (No. 1). The collected extracts were finally concentrated using a rotary evaporator (RE-300DB evaporator, Stuart) set at 40˚C. Then, they were stored at 4˚C for the phytochemical analysis. Finally, each dry residue was dissolved in the corresponding solvent to make a solution of 1 mg/mL. These solutions were used for the phytochemical screening tests.

2.3.4. Preliminary Phytochemical Screening

Based on Venkatesan et al. [29], Adetuyi and Popoola [30], Trease and Evans [31], Sofowora [32], Harborne [33], Sofowara [34], and almasehali [35], the following tests were carried out in each extract to determine the presence of selected phytochemical classes with potential biological activity.

2.3.4.1 Test of Alkaloids

A weight of 0.2 g of each extract was mixed with 2% sulphuric acid in a separate test tube and warmed for 2 minutes. The mixture was then filtered in a new test tube. A few drops of Dragendorff's reagent were added. The formation of orange or reddish-brown precipitates indicates the presence of alkaloids.

2.3.4.2 Test of Flavonoids

A weight of 0.2 g of each extract was dissolved in dilute sodium hydroxide, followed by the addition of dilute hydrochloric acid. A disappearing of the yellow coloration confirms the presence of flavonoids.

2.3.4.3 Test of Saponins

A weight of 0.2 g of each extract was mixed with 20 ml of distilled water and boiled and filtered. About 10 ml of the filtrate was then transferred to a new test tube and mixed with 5 ml of distilled water and gently shaken. The formation of a stable, persistent froth. A few drops of olive oil were then added and shaken vigorously. The test is positive for saponin if frothing appears as a creamy cluster of small bubbles.

2.3.4.4 Test of Reducing Sugars

An aliquot of 2 ml of each extract was transferred to a separate test tube, diluted in 5 ml of distilled water, and filtered. A few drops of Fehling's solutions A and B were added to the filtrate and boiled for 2 minutes. The observation of an orange-red precipitate indicates the presence of reducing sugars.

2.3.4.5 Test of Cardiac Glycosides

A 5 ml aliqout of each extract was treated with 2 mL of glacial acetic acid mixed with one drop of ferric chloride solution. This was followed by careful addition of 1 mL of concentrated sulphuric acid carefully. The formation of a brown ring at the interface indicates a deoxysugar characteristic of cardenolides. Similary, a violet ring may also appeare just below the brown ring while in the acetic acid layer, in the same manner a greenish ring may appear just gradually throughout the thin layer.

2.3.4.6 Test of Terpenoids

A weight of 0.5 g of each extract was mixed with 2 ml of chloroform in a separate test tube. Concentrated sulphuric acid was then added along the side of the test tube carefully to form a layer. The tube was then observed for the appearance of reddish brown coloration in the interface, which may be formed when terpenoids are present.

2.3.4.7 Test of Carbonyl

A quantity of 2 ml of each extract was transferred to a separate test tube, and few drops of 2,4-dinitrophenylhydrazine solution were added. The tubes were shaken and observed immediately for the formation of yellow crystals, which denotes the presence of carbonyl.

2.3.4.8 Test of Phenols and Tannins

A quantity of 0.5 g of each extract was mixed with distilled water and heated in a water bath. This mixture was then filtered and ferric chloride solusion was added. The tubes were visually inspected for the presence of dark green or a blue-black coloration, which indicates the presence of phenols/tannins.

2.3.4.9 Test of Phlobatanin

A weight of 0.5 g of each extract was dissolved in distilled water and filtered. A 2% hydrochloric acid solution was added to the filtrate and boiled. The formation of a red precipitate confirms the presence of phlobatanin.

Table 1. Preliminary phytochemical screening of ethanol extracts of Pulicaria jaubertii plant parts and callus

Phytochemical	Leaves	Stems	Roots	Flowers	Callus
Alkaloids	+	+	+	+	+
Flavonoids	+	+	+	+	+
Saponins	+	+	+	+	+
Reducing sugars	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+
Terpenoids	+	+	+	+	+
Carbonyls	+	-	+	+	+
Phenols/Tannins	+	+	+	+	+
Phlobatanin	+	+	-	+	+
Steroids	+	+	+	+	+

Table 2. Preliminary phytochemical screening of n-hexane extracts of Pulicaria jaubertii plant parts and callus

Phytochemical	**Leaves**	**Stem**	**Root**	**Flower**	**Callus**
Alkaloids	-	-	-	-	-
Flavonoids	+	-	-	+	-
Saponin	-	-	+	-	-
Reducing sugars	-	-	+	-	-
Cardiac glycosides	+	+	+	+	+
Terpenoids	+	+	+	-	+
Carbonyl	-	-	+	+	+
Phenols/Tannins	-	+	-	-	-
Phlobatanin	-	-	-	-	-
Steroids	+	+	+	+	+

2.3.4.10 Test of Steroids

Two milligrams of each dried extract were dissolved in acetic anhydride, boiled, cooled, and then 1 ml of concentrated sulphuric acid was carefully added along the sides of the test tube. The mixture was then observed for the color change from violet to blue or green, which indicates the presence of steroids.

3. RESULTS

Table 1 shows the results of the phytochemical profiling of preliminary screening tests of the ethanol extracts, which reveal the presence of alkaloids, flavonoids, saponins, reducing sugars, cardiac glycosides, terpenoids, phenols/tannins, and steroids in all the plant parts and the callus. However, carbonyl compounds were identified in all the plant parts except the stems, and phlobatanins were detected in all parts except the roots. In the callus, both carbonyl compounds and phlobatanins were also detected.

Table 2 shows the results of the preliminary screening tests of the

phytochemicals in the n-hexane extracts, which indicated the presence of cardiac glycosides and steroids in all the plant parts and the callus. Terpenoids were also recorded in the callus and all the plant parts except the flowers. Carbonyl compounds were detected in the callus, roots, and flowers. Saponins and reducing sugars were detected only in the roots, and flavonoids were identified only in the leaves and flowers. Phenols/tannins were absent in all the plant parts except the stems. Contrariwise, alkaloids and phlobatanins were not found either in the plant parts or in the callus.

4. DISCUSSION

In the present study, ethanol extracts of *P. jaubertii* showed the presence of various phytochemical constituents like alkaloids, flavonoids, saponins, reducing sugars, glycosides, terpenoids, phlobatanins, carbonyls, phenols/tannins, and steroids in almost all the plant parts and the callus (Table 1). On the other hand, n-hexane extracts showed the presence of most of these phytochemicals, but some of these constituents were absent in some of the plant parts and the callus (Table 2). Unlike ethanol, n-hexane is known as a nonpolar solvent, and

most of the polar compounds are not dissolved in it. However, the presence of these phytochemical groups in the extracts was in agreement with other studies that have reported the presence of many of these compounds in the EO extracted by hydrodistillation of the areal parts of this plant [5–8]. These results were also observed in various extracts of other species of *Pulicaria*, such as *P. incise*[8–11, 34]; *P. crispa* [13, 16]; *P. undulata* [13–15]; and *P. mauritanica* [16]. Other genera belonging to the same family, Asteraceae, were also reported to contain many of these phytoconstituents, in which leaves and stems of *Anvillea Radiata*, *Cotula cinerea*, and *Matricarea pubescens* were found to contain the saponins, terpenoids, alkaloids, tannins, flavonoids, and steroids [36]. Equally, the phytochemical screening of *Asteriscus graveolens* (family: Asteraceae) revealed the presence of alkaloids, coumarins, condensed tannins, terpenes, and cyanogenic compounds [37].

Furthermore, the ethanol extract of the callus showed the presence of all the investigated phytochemical groups in this study. Nevertheless, to our knowledge, studied involving *in vitro* propagation of *P. jaubertii*, and the identification of bioactive substances in the callus has not yet been performed. Likewise, not many studies were conducted to identify the phytoconstituents in the callus of the plants belonging to the *Pulicaria* genus and Asteraceae family. The only recognized published study on other *Pulicaria* species was carried out by Rouane *et al.* [38], who reported that mucilage, saponins, flavonoids, and anthocyanins were detected in the callus of *P. incise*. Nonetheless, many of these phytochemicals revealed in the callus in this study were also detected in the callus of other various plant species belonging to other families, such as *Solanum nigrum* (family: Solanaceae) [39]; *Corynandra felina* (family: Cleomaceae) [40]; *Passiflora caerulea* (family: Passifloraceae) [41]; and *Mussaenda glabrata* (family: Rubiaceae) [42].

Interestingly, *P. jaubertii* extract was reported to have potent antimicrobial, antioxidant, and anti-inflammatory properties [4, 7, 43] and various other therapeutic activities [3]. These pharmacological effects may be associated with the presence of these phytochemicals in this plant. It is known that various groups of the plant's secondary metabolites such as flavonoids, alkaloids, phenols, glycosides, terpenoids, and volatile oils, are used as protective and immune-supportive agents against infectious pathogens [44]. In this study, flavonoids were found in all the plant parts and the callus. Flavonoids have been reported to exhibit anti-microbial, anti-allergic, anti-inflammatory, anti-oxidative, and anti-cancer activities, as well as a protective anti-ulcer property on the mucosa [45–48]. Alkaloids were also detected in all the plant parts and the callus. Alkaloids are have been reported to act as chemopreventive phytochemicals to suppress cancer growth and tumor development [49]. Similarly, reducing sugars were detected in every part of the plant as well as in the callus. The presence of carbohydrates in the plant could provide the energy required to enhance immune system activities [50]. Terpenoids, saponins, phenols/tannins, phlobatanins, glycosides, and steroids were also shown to be present in most of the plant parts and in the callus. As suggested [1, 51], terpenoids extracted from some *Pulicaria* species have antimicrobial actions. Terpenoids also have antitumor, anticancer, anti-inflammatory, anti-malarial, analgesic, and anti-cardiovascular disease properties [52]. They showed the ability to relax cardiovascular smooth muscle by several mechanisms [53]. Furthermore, phenols were found to reduce blood cholesterol levels and protect against coronary heart disease and some types of cancer [54]. Tannins were claimed to have cytotoxic and anti-tumor activities [46], and they could be used in the treatment of ulcerations, inflammation, and intestinal disorders including diarrhea and dysentery [55]. Similarly, as mentioned by Olivia *et al.* [56], saponins and tannins may reduce the inflammation, protect the gastric mucosa, and reduce its acidity. This protective action against peptic ulcer could also be exerted by terpenoids and alkaloids [57, 58]. Phlobatannins were reported to have wound healing, anti-inflammatory, analgesic, and antioxidant properties [59]. Cardiac glycosides are known to be medically used as a cardiotonic and to treat renal and infectious diseases [54]. Cardiac glycosides were also found to have sedatives and antispasmodic properties [60]. Plant steroids were reported to have immunosuppressive, hepatoprotective, cytotoxic, anti-tumor, plant growth hormone regulator, sex hormone, cardiotonic antibacterial, and antihelminthic activities [61].

From this perspective, the presence of these phytochemical classes in both plant parts and callus highlights the importance of conducting further studies involving other solvents and more sophisticated separation techniques to accurately characterize their bioactive constituents and to discover and develop new therapeutic drugs from this plant.

5. CONCLUSION

In this study, which involves the screening of phytochemical classes in the ethanol and n-hexane extracts of the plant parts and the callus of *P. jaubertii*, several phytochemical classes were detected in both extracts, particularly the ethanol extracts for which the therapeutic properties of many of these phytochemical groups are well known. Nevertheless, the identification of the phytocompounds in the species belonging to these groups and their therapeutic activities has yet to be fully elicited. Further phytochemical and pharmacological studies, including developed separation techniques and various solvents, are recommended.

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