DECIPHERING ANTIFUNGAL POTENTIAL OF HIMALAYAN MARSH ORCHID- DACTYLORHIZA HATAGIREA

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Abstract

Fungal infections are a serious global health concern, contributing significantly to morbidity and mortality rates. The emergence of antifungal resistance and the relative toxicity of existing medications have made it necessary to explore alternative treatments, such as herbal medicines used in traditional folk therapies. This study aims to evaluate the in vitro antifungal activity of *Dactylorhiza hatagirea*, a Himalayan orchid. The roots of the plant were collected and extracted using a hydroalcoholic solvent. Qualitative and quantitative analyses were performed, and antifungal activity was assessed. The results showed the presence of flavonoids, phenols, proteins, carbohydrates, saponins, and tannins. The total phenol and flavonoid content were found to be 0.496mg and 0.784mg/100mg, respectively. The extract with a concentration of 100mg/ml demonstrated a zone of inhibition of 13±0.86mm against Candida albicans and 10±0.94mm against Aspergillus flavus. Therefore, the study concludes that the roots of *Dactylorhiza hatagirea* possess potent antifungal activity.

Keywords: *Dactylorhiza hatagirea*, Antifungal activity, Medicinal plants, *C. albicans*, *Aspergillus flavus*, Herbal medicines

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Introduction

Fungal infections pose a significant risk to public health, with a range of severity levels that include mucosal, systemic, cutaneous, subcutaneous, and superficial infections. Candida spp. is one of the organisms present in the human microbiota that can cause opportunistic infections in healthy individuals and potentially fatal infections in immunosuppressed patients, such as those with HIV, cancer patients receiving chemotherapy, and people taking immunosuppressive medications. Patients with underlying conditions may also develop healthcare-associated infections from fungal pathogens such as Candida, Aspergillus, Fusarium, Mucorales, and moulds (Perlroth et al., 2007; Lee and Lau,2007).

Fungal infections are a significant global health issue and can affect different parts of the body, including the skin and mucosal surfaces. These types of infections are particularly problematic in developing countries, especially in tropical and subtropical regions. Impaired immune systems are often associated with fungal infections, and certain groups of fungi, such as dermatophytes and Candida species, are commonly responsible for skin infections. Candida albicans and related species can cause oral and vaginal candidiasis, which is prevalent in adult women who have experienced at least one episode of vulvo-vaginal candidiasis. Dermatophytosis is the most contagious and widespread fungal disease, and the prevalence of this condition has increased significantly in recent years. Dermatophytes thrive in humid, overcrowded environments with poor hygiene, and they infiltrate keratinized tissues. (Perlin et al., 2017; Velayuthan et al., 2018).

There is a need to conduct an evaluation and chemical investigation of the active extracts from various medicinal plants that are believed to have anti-fungal properties. This is necessary to identify the secondary metabolite that is inherent in these plants, as drug-resistant fungal pathogens are widespread and pose a significant threat. Moreover, chemically sourced anti-fungal agents have limited success in containing these drug-resistant strains. Therefore, exploring herbal medicines from traditional folk therapies may provide a promising alternative due to their low cost and lack of side effects. Plant extracts have historically been used to treat various infectious disorders, including those caused by bacteria, fungi, protozoa, and viruses, and are expected to be an important source of new therapeutic molecules (Mohammadi et al., 2014; Hamza et al., 2006).

The problem of antifungal drug resistance and the associated toxicity can potentially be addressed by utilizing traditional herbal medicines. Herbal medicines have been historically used in various cultures to treat a variety of infectious diseases caused by bacteria, fungi, viruses, and protozoa, and they are increasingly popular due to their low cost and minimal side effects. In addition, plant extracts may provide a valuable source of new therapeutic compounds. Thus, it is important to investigate and analyze the active extract of medicinal plants that are believed to have antifungal properties to identify the secondary metabolite responsible for their activity (Mohammadi et al., 2014; Hamza et al., 2006).

The effectiveness of plant extracts against human and plant pathogens that cause fungal infections has been widely studied. In particular, medicinal herbs commonly used in Indian traditional medical systems have been evaluated for their potential antimicrobial properties. To further explore this, a study was conducted to determine the antifungal activity of *Dactylorhiza hatagirea*, a Himalayan orchid native to India. This study evaluated the in vitro effectiveness of the plant extract against clinical fungal isolates.

Materials & Methods

Procurement of Plant Material

Roots of *Dactylorhiza hatagirea* were collected from local market of Bhopal, month of January, 2022. After the plant was collected they have been processed for cleaning in order to prevent the deterioration of phytochemicals present in plant.

Powdering

The dried plant part was finely powdered using electric grinder, sieved and packaged in polyethylene bags until when needed.

Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs (Khandelwal, 2005; Kokate, 1994)

Degreasing of plant material

150 gram of dried powdered Roots of *Dactylorhiza hatagirea* was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted dried powdered roots of *Dactylorhiza hatagirea* has been extracted with hydroalcoholic solvent (80:20: ethanol: water) using maceration

process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C.

Qualitative phytochemical analysis

Phytochemical screening was performed as per standard method.

Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Gaur Mishra *et al.,* 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Parkhe and Bharti, 2019). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

In-vitro Anti fungal activity using well diffusion method

In vitro antibacterial activity was performed on isolated colonies of *C. albicans* & *A.niger*. The Agar well diffusion technique was used for determining the zone of inhibition and minimum inhibitory concentrations (MIC). The strains of *C. albicans* & *A.niger* were inoculated in prefabricated agar plate. Plates were dried and 4 wells were made with the help of 6 mm agar well cutter. 25 mg/ml, 50 mg/ml, 100 mg/ml of prepared extract concentrations was loaded in all the respective wells. The agar plates were kept undisturbed to allow the passive diffusion of extracts into the agar culture medium. Then the plates were incubated at 37°C for 24 hours. The zone of inhibition was calculated in mm (Afolayan *et al*., 2002).

Result & Discussion

Dactylorhiza hatagirea, also known as Himalayan orchid or Salam Panja, is a medicinal plant that is native to the Himalayan region. The plant is known for its medicinal properties and is used in traditional medicine to treat various ailments. *Dactylorhiza hatagirea* collected from the wild or cultivated in a controlled environment. The collected plant material should be authenticated to ensure that it is indeed *Dactylorhiza hatagirea* and not a different plant species. Authentication can be done by comparing the morphological characteristics of the plant, such as the shape and size of roots, with the authentic reference material. Once the plant material is collected and authenticated, it can be dried for further use. Drying can be done using natural method, such as shade drying. It is important to dry the plant material at a suitable temperature and humidity to prevent the degradation of active compounds and to maintain the quality of the plant material.

During the drying process, the plant material should be protected from direct sunlight, moisture, and contamination to prevent microbial growth and chemical degradation. Once dried, the plant material should be stored in airtight containers in a cool and dry place to maintain its quality and shelf life.

The extraction of *Dactylorhiza hatagirea* roots using a hydroalcoholic solvent (Ethanol: water, 80:20v/v) is a commonly used method to isolate the bioactive compounds from the plant material. The hydroalcoholic solvent is preferred as it can extract a wide range of compounds with varying polarities. The hydroalcoholic extraction method can be an efficient method to isolate the bioactive compounds from Dactylorhiza hatagirea roots. The extracted material can be further analyzed for its phytochemical composition and biological activity.

The determination of percentage yield is an important parameter to evaluate the efficiency of the extraction process of *Dactylorhiza hatagirea* roots. The percentage yield can be calculated by dividing the weight of the extracted material by the weight of the dried plant material and multiplying by 100. The percentage yield can be affected by various factors and can be used to optimize the extraction conditions and evaluate the reproducibility of the extraction process. The percentage yield was found to be highest in hydroalcoholic solvent which is about 8.33% while in case of pet ether it was found to be 4.75% table 1.

Phytochemical screening of the hydroalcoholic extract of *Dactylorhiza hatagirea* roots is an important step to identify the presence of various secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds. The phytochemical screening revealed the presence of flavonoid, phenol, protein, carbohydrate and saponin table 2.

The total phenol and flavonoid content in the hydroalcoholic extract of *Dactylorhiza hatagirea* roots can provide information about the potential antioxidant activity of the extract. Quantification method: The quantification of total phenol and flavonoid content can be done using various methods such as the Folin-Ciocalteu method for phenol and the aluminum chloride method for flavonoids. The quantification method used should be validated and standardized to ensure accurate and reproducible results.

The total phenol and flavonoid content can be correlated with the antioxidant activity of the extract. A higher content of phenols and flavonoids can indicate a higher antioxidant activity of the extract. The total phenol and flavonoid content can have biological significance as these compounds have various health benefits such as anti-inflammatory, anti-cancer, and anti-diabetic activities. Therefore, the quantification of these compounds can provide an indication of the potential therapeutic value of the extract. The total phenol & flavonoid content was found to be 0.496mg & 0.784mg/ 100 mg respectively. The determination of the total phenol and flavonoid content in the hydroalcoholic extract of *Dactylorhiza hatagirea* roots can provide valuable information about the potential antioxidant activity and therapeutic value of the extract table 3.

The antifungal activity of the hydroalcoholic extract of *Dactylorhiza hatagirea* roots against *A. niger* and *C. albicans* can provide valuable information about the potential therapeutic value of the extract against fungal infections. The antifungal activity of the extract determined using well diffusion method.

The antifungal activity of the extract compared with standard fluconazole. The comparison based the zone of inhibition of the extract and the standard drug. The extract with concentration of 100mg/ml found zone of inhibition of 13±0.86 mm in case of *C. albicans* and 10±0.94 for *Aspergillus flavus*. When the zone of Inhibition compare with standard drug fluconazole found zone of inhibition 32±0.74mm and 30±0.5 against *C. albicans* and *Aspergillus flavus* at the concentration of 30µg/ml. The antifungal activity of the extract attributed to the presence of various phytochemicals such as pHenols and flavonoids. The antifungal activity of the extract can have significant biological and therapeutic significance as fungal infections can cause various diseases and can be difficult to treat. Therefore, the antifungal activity of the extract can provide valuable information for the development of new antifungal drugs. The results of the experiment can provide valuable information about the potential therapeutic value of the extract against fungal infections table 4 & 5.

Table 1: Extractive values of *Dactylorhiza hatagirea*

S. No.	Extracts	% Yield* (W/W)
1.	Pet. ether	4.75 %
2.	Hydroalcoholic	8.33%

Table 2: Result of phytochemical screening of extract of *Dactylorhiza hatagirea*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Hager's Test:	 -ve
2.	**Glycosides** Legal's Test:	 -ve
3.	**Flavonoids** Lead acetate Test: Alkaline test:	 +ve +ve
4.	**Diterpenes** Copper acetate Test:	 -ve
5.	**Phenol** Ferric Chloride Test:	 +ve
6.	**Proteins** Xanthoproteic Test:	 +ve

7.	**Carbohydrate** Fehling's Test:	+ve
8.	**Saponins** Froth Test:	+ve
9.	**Tannins** Gelatin test:	+ve

Table 3: Estimation of total phenol and flavonoids content of *Dactylorhiza hatagirea*

S. No.	Extract	Total phenol content	Total flavonoids content
1.	Hydroalcoholic	0.496mg/ 100 mg	0.784mg/ 100 mg

Table 4: Antimicrobial activity of fluconazole against *Candida albicans* and *Aspergillus flavus*

S. No.	Microbes	Zone of inhibition		
		10µg/ml	20µg/ml	30µg/ml
1.	*Candida albicans*	23±0.5	30±0.47	32±0.74
2.	*Aspergillus flavus*	17±0.47	24±0.57	30±0.5

Table 5: Antimicrobial activity of Hydroalcoholic extract against *Candida albicans* and *Aspergillus flavus*

S. No.	Microbes	Zone of inhibition		
		25mg/ml	50mg/ml	100mg/ml
1.	*Candida albicans*	7±0.57	11±0.94	13±0.86
2.	*Aspergillus flavus*	7±0.74	9±0.5	10±0.94

Conclusion

In comparison to traditional medications, the hydroalcoholic extracts of *Dactylorhiza hatagirea* were found to be active on isolated fungus. The current investigation supported the claimed use of roots in conventional medicine for the management of several infectious diseases brought on by the fungi *A. niger* and *C. albicans*. However, additional research is required to more accurately assess the potential efficacy of the crude extracts as the antibacterial agents. The current findings will serve as the foundation for choosing plant species for additional study in the hope of discovering novel natural bioactive chemicals. There has to be more research done to isolate and understand the chemical makeup of the plant's antifungal active components.

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