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Potentializing Partially Purified Bioactive Phytochemical for the Therapeutics of Fungal Dermatophytosis

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Abstract
Fungal infection are the most common causes of superficial or cutaneous infections around the world and remain a major public health problem in spite of the presence of various antifungal drugs. The difficulties associated with the existing prolonged treatment of fungal infection and antifungal drugs resistance remain challenges to select an effective antifungal agent. Plants have medicinal properties due to bioactive phytochemicals that can be potentially explored for the curing of fungal skin infections. Their use as herbal formulations against fungal infection is one of the safe alternative treatments instead of synthetic drugs. Phytochemicals as secondary constituents contain alkaloid, flavonoid, saponin, phenol, and tannin are more potent with respect to their crude counterpart. The present study aims to explore two medicinal plants as *Catharanthus roseus,* and *Tagetes erecta* locally available in Krishi Vigyan Kendra (KVK) Banasthali Vidyapith using solvents as water and methanol.

Keywords: Medicinal plants, Fungal infection, Phytochemicals.

1. Introduction

There are two categories of fungal infections: primary and opportunistic. Although primary infections can also happen in hosts with a healthy immune system, opportunistic infections primarily affect immune-compromised hosts [1]. In addition, fungal infections might be local or systemic. According to estimates, between 984 million and 1 billion people would be affected by serious fungal diseases between 1984 and 2021, with fungal skin infections being the cause of over a million fatalities annually [2]. According to reports, fungi infections generate high rates of morbidity and mortality [3]. The keratinous fungi are unique in that they may feed on keratin-rich substances found in soil or in the tissues of people and animals, including their skin, hair, and nails [4]. Taxonomically, dermatophytes fall within the family Arthrodermataceae, which has seven genera, and the order Onygenales. Only three genera, *Microsporum*, *Trichophyton*, and *Epidermophyton*, are, however, frequently linked to dermatophytosis in both humans and animals [5]. They can affect any region of the human body's skin, giving rise to names like tinea unguium (nails), tinea capitis (scalp), tinea corporis (body), tinea cruris (groyne), and tinea pedis (feet), depending on the affected area [6]. According to their natural habitats, they can be divided into three groups: anthropophilic species (which can only infect people), zoophilic species (which can infect both animals and humans), and geophilic species (able to cause humans after contact with contaminated soil) [7]. Plants one of the richest, oldest and most varied cultural traditions involving the use of medicinal

herbs is found in India. Traditional knowledge, which can be found in the forms of Unani, Siddha, Ayurveda, and Swa-riga (Tibetan) systems of medicine, serves as the foundation for modernized medical practices [8]. Plant extracts from plants that contain a complex mixture of different bioactive components (alkaloid, flavonoids, saponins, tannin and phenolic) are widely used to make plant-derived medications, which are used to treat both chronic and infectious disorders because they have anti-fungal in medicinal plants [9]. The current study aims to assess the bio efficacy of herbal and herbonanoconjugate as safe therapy of fungal dermatophytic infection and the antifungal efficacy of partly purified phytochemicals obtained from plants. The objective of the current study was to compare antifungal efficacy.

2. Materials & Methods

Collection of Plant Material

These wild medicinal plants were chosen for conducting the study on the basis of cost effectiveness, ease of availability and medicinal properties. These include *Catharanthus roseus (Sadabhar), and Tagetes erecta (Gainda),* Leaves of plants were collected from the campus of Krishi Vigyan Kendra (KVK) Banasthali Vidyapith, Jaipur, Rajasthan. Fresh leaves were collected washed thoroughly 2-3 times with running tap water and once with sterile distilled water remove adhering organic contaminants and then air dried at room temperature, dried samples were ground (Croma 500 W Mixer grinder *CRAK4184*) to make fine powder which was stored in air-tight containers for further use.

Solvent extraction: The dried leaf powdered was performed to aqueous and methanol extracts using the Soxhlet method for plant extracts. Twenty grams of leaf powdered was filled in the thimble and then successively extracted by aqueous and methanol for 48 hours at 55°C. Using a rotary flash evaporator with reduced pressure, all of the solvent extracts were concentrated. Individual plant leaves were dried, and the leaf powder was utilized for solvent extraction by the Soxhlet method using aqueous and methanol. The extracts were stored in airtight brown bottles until further use. The plant extracts were then used to characterize the phytochemicals and test for antifungal efficacy.

Extraction of Phytochemicals Associated with the Test Plants

The plant extracts aqueous and methanolic were then used to extract the plant's active metabolites (alkaloids, flavonoids, saponin, tannin, and phenolics) followed by their qualitative and quantitative analysis. The chemical structures of below isolated partially purified phytochemicals are shown in figure.

Qualitative analysis: Determination of the phytochemical constituents the extract was evaluated for the preliminary phytochemical screenings were carried out on the crude extracts as described to identify the presence of the classes of secondary metabolites (Alkaloids, flavonoids, tannins, saponins, and phenol).

Qualitative test for alkaloids: Few drops of sample plant extracts were stirred with 5ml of 2M $HCl_{(aq.)}$ on a heated-on water bath for 5 minutes after that filtrate (1ml) solution added few drops of Drangendoff's reagent (Figure A). The result solution was observed for color change. An orange red precipitate was delivery red quickly, which showed the presence of alkaloids [10].

Qualitative test for flavonoids: Take a small amount of the plant extract in a test tube. Add a few drops of 10% sodium hydroxide solution. Observe the colour change. The formation of a yellow color, which disappears on the addition of dilute hydrochloric acid, (Figure B) indicates the presences of flavonoids [11].

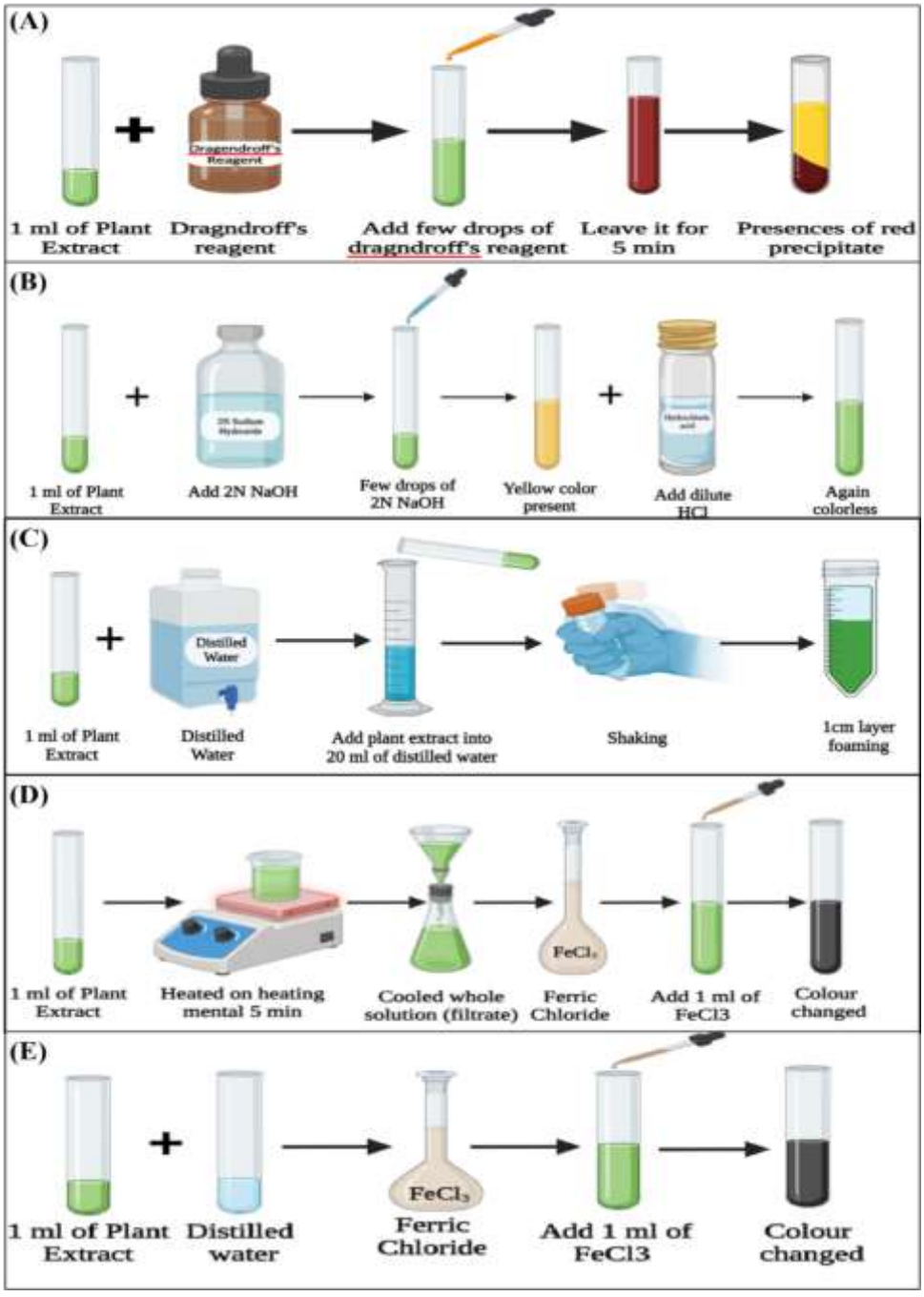
Qualitative test for saponins: The presence of saponins was determined using a foam test. Each plant extract was combined in small amounts with 20 ml of distilled water in a 100 ml beaker. The mixture was then heated and filtered, with the filtrate being utilised for the Froth test. 5 ml of the filtrate diluted with

water (20 ml), after being forcefully shaken and was let to stand for 30 min (Figure C). The result was recorded [12].

Qualitative test for tannins: Few drops of sample plant extracts were heated on water bath with distilled water for 5 minutes after that cooled whole solution (filtrate) after that 1 ml of $FeCl_3$ was added (Fig. D). The color change noted [13].

Qualitative test for Phenols: 2ml of water and few drops of 10% aqueous ferric chloride solution are added to 1 ml of each extract for the test (Figure E). One can see the blue or green coloring [14].

Figure 1. Flowchart showing the presence of extraction of phytochemicals associated with the test plants (qualitative): (A) presence of alkaloids (B) presence of flavonoids (C) presence of saponin (D) presence of tannin (E) presence of phenol



3. Results

The present study aims to evaluate the efficacy of the test plants and their bioactive phytochemicals (partially purified). In the current research these bioactive phytoconstituents were bioactive compounds presents.

Screening of Phytochemicals/Plant's Active Metabolites

Qualitative Analysis:

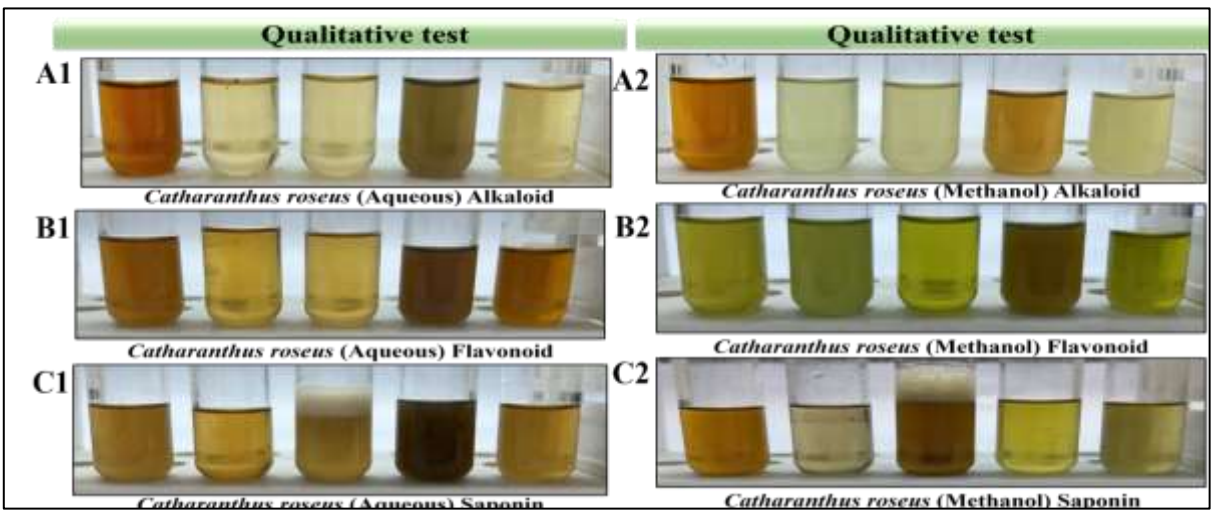
The phytochemical screening of plant extracts aqueous and methanolic extracts samples revealed the presence of some secondary metabolites such as flavonoids, saponin alkaloids, tannin, and phenol as shown in Table 1. The phytochemical compounds detected are known to have medicinal importance. These bioactive compounds have been reported as powerful poison and many phytochemicals derived from medicinal plants show biological activities like, anti-fungal.

Table 1. Qualitative test for the all the test plants extract

Phyto- constituent	Phytochemicals test	Plants Name	Aqueous	Methanol
Test for Alkaloid	**Dragendorff's test**	*Catharanthus roseus*	++	+
		Tagetes erecta	++	++
Test for Flavonoids	**Lead Acetate test**	*Catharanthus roseus*	+++	++
		Tagetes erecta	+++	++
Test for Saponin	**Foam test**	*Catharanthus roseus*	++	+++
		Tagetes erecta	++	++
Test for Tannin	**Ferric Chloride test**	*Catharanthus roseus*	+++	++
		Tagetes erecta	++	+
Test for Phenol	**Ferric Chloride test**	*Catharanthus roseus*	+	+
		Tagetes erecta	+++	+++

***(+) present and (-) not present**

Figure 2. Photo plates showing the presence of extraction of phytochemicals (bioactive compounds) associated with the test plants (qualitative): *Catharanthus roseus/Tagetes erecta* (A1:A2) presence of alkaloids; (B1:B2) presence of flavonoids; (C1:C2) presence of saponin; (D1:D2) presence of tannin; (E1:22) presence of phenol respectively.



4. Discussion

Medicinal plants contain a wide variety of secondary metabolites which has subjected several plants of medicinal importance to screening in a bid to discover new drugs that can be effective in the treatment of various diseases including dermatophytosis [15]. The medicinal plants bioactive constituents generate a distinct physiological impact on the human body. The phenolic compounds, flavonoids, alkaloids, tannins, and saponins are the most significant of these chemically active plant components. Plant extract phytochemicals are recognized to have physiological and therapeutic effects. It was observed in this study that the aqueous and methanolic plant extracts of two wild medicinal plants contain various phytochemicals known to exhibit a variety of biological activities such as anti-dermatophytes [16] and these phytocompounds were reported to have different modes of actions.

The qualitative results of the phytochemical analysis of aqueous and methanolic extract of wild medicinal plants revealed the presence of alkaloid, flavonoids, saponin, tannins, and phenols. Show the absence of essential oils. From this result, it can be shown that the presence of these active compounds was responsible for the anti-dermatophytic effects exhibited by leaf plants extracts. This result corresponds

with the epidemiological investigation by Oyerinde et al. (2013) [17] who reported that the presence of these phytochemicals may be responsible for the bioactive properties of the extracts [18].
Negri et al., 2014, [19] reported the antifungal effect of tannins from *Tagetes erecta* on skin fungi. The tannins present, along with other constituents detected in the present study could be responsible for the observed high growth in inhibition. Tannins are naturally occurring plant polyphenols whose primary property is their ability to precipitate and bind proteins. They can significantly affect the nutritional content of many foods consumed by people and the feed consumed by animals. Studies have demonstrated that tannins have antifungal properties. The synergistic effect as antifungal agents of several constituents has been reported in several plant species [20].
As a result, the plants extracts were isolated and purified for key phytochemicals that may be effective in the treatment of a variety of ailments. The medicinal values of these plants lie in their component phytochemicals, which may produce definite physiological actions on the human body.

5. Conclusion

Fungal skin conditions affect people of all ages, including infants and the elderly, and are frequently brought on by bacteria, viruses, and fungus. In this situation, fresh ideas and innovative experimental methods are needed to treat skin issues in persons with nonfatal disabilities. Despite the fact that more than 50% of plant species are utilized to cure skin conditions, many of them are susceptible to extinction due to human activities such habitat degradation, deforestation, and urbanization. Many fungal species are also resistant to routinely used medications, and this resistance has mostly increased in recent years. Invaluable sources of potent antifungal and therapeutic chemicals, medicinal plants may be used to treat a variety of illnesses, including fungal infections. Plant-based substances could serve as an alternative to conventional medications. Although there are many different possible mechanisms of action that depend on the primary chemicals in the plant extract, the therapeutic impact largely centers on the synergistic activity of these compounds. *In vitro* and *in vivo* researches have revealed that plant extracts have inhibitory effects on the development of fungus. Furthermore, natural substances may impair viral attachment and penetration into the host by interfering with the fungal resistance systems.

6. CONFLICT OF INTEREST

Disclosure of potential conflicts of interest indicates that there is no conflict of interest during the study conduct in any of the authors. The study is conducted in compliance with Ethical Standards.

7. ACKNOWLEDGEMENT

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8. AUTHOR CONTRIBUTION

"All authors have read and approved the final version of the manuscript [Sarika Gupta or Anusha Sharma] had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis."
This work was carried out in collaboration between both authors. Author SG provided guidance during the study conduct, designed the study, performed the statistical analysis, and standardized the protocol.

Author AS wrote the first draft of the manuscript, managed the analyses of the study through managing the literature searches. Both authors read and approved the final manuscript.

9. TRANSPARENCY STATEMENT

Sarika Gupta or Anusha Sharma affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained".

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