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## Novel phytotherapy for tinea versicolor by extracting *Zingiber wightianum* Thwaites

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### Abstract

Herbal medicines are gaining more interest and patient compliance largely due to the fact that it has increased medicinal and economic benefits when compared to that of allopathic medications in addition to that, they are also shown to exhibit lesser side effects. The present research was aimed at phytochemical screening and antifungal evaluation of rhizomic extract of *Zingiber wightianum* Thwaites belonging to Zingiberaceae family. The rhizomes were initially extracted using a Soxhlet apparatus and then preliminary phytochemical screening of the extract was further conducted. Upon analysis, the extract proved the presence of alkaloids, carbohydrates, tannins, flavonoids, saponins, phenols. Subsequently, these extracts were furthermore assessed for both antifungal activity and minimum inhibitory concentration. Hence the potential to treat a fungal infection Tinea versicolor, caused by fungus *Malassezia furfur* was revealed. This investigation was also undertaken with a view to formulate and evaluate the herbal gel using *Zingiber wightianum* rhizomic extract. Thus, the overall studies concluded that the rhizomic extract of *Zingiber wightianum* Thwaites and its formulation would be a breakthrough in the field of modern antifungal drug.

**Keywords:** *Malassezia furfur*, *Zingiber wightianum* Thwaites, Anti-fungal, Tinea versicolor, Phytochemical Analysis.

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### Introduction

Many plants are traditionally used as folk medicine for the treatment of a wide range of infectious disease, which sums up for the reason why investigations on the chemical compounds found in various plants have become desirable. In addition to which widespread resistance to antibiotics, the emergence of novel pathogens, resurgence of old ones and the deficiency of effective modern therapeutics adds fuel to the fire. Thus, the discovery and development of new antimicrobial agents from natural plant extracts is essential. In the present work, we develop a new herbal drug for the

treatment of Tinea versicolor and thus investigates the capability of *Zingiber wightianum* to act as an antifungal agent against *Malassezia furfur*.

*Zingiber wightianum* is a monocotyledonous plant belonging to the family Zingiberaceae. Its synonym is mountain ginger. Ample number of genera and species belonging to the Zingiberaceae family are spread throughout the tropical and subtropical regions, out of which *Zingiber wightianum* is the least researched plant when it comes to both investigations of its chemical composition and well as its biological properties [5].

Pityriasis versicolor, commonly referred to as Tinea versicolor is a frequent, benign, superficial, fungal infection of the skin. It is caused by a type of yeast that naturally lives on your skin. It can be attributed as a mycotic infection of the skin caused by a dermatophyte *Malassezia furfur* (*Pityrosporum ovale*). Tinea versicolor is characterized by hyper pigmented or hypo pigmented finely scaly macules mainly seen on the trunk, neck and proximal extremities. The spot may disappear during

cool weather and worsen during warm and humid weather. These are likely to be dry and scaly and may itch or hurt. This disease is diagnosed by ultraviolet light which appear as fluorescent yellow green on its exposure [1-4]. *Malassezia furfur*, earlier known as *Pityrosporum ovale* is a lipophilic fungus belonging to the pleomorphic yeast category. These fungi are most likely to be found at the superficial layer of the skin (stratum corneum) as well as the hair follicles. As fat is essential for its growth and development, areas of the skin having many sebaceous glands provides home to the fungus. This yeast is found in more than 90% of adults without causing any infection. But tinea versicolor is caused by its unusual growth in the skin [6-9].

From the investigations, ethanolic extract proved its antifungal potential to treat *Tinea versicolor* against *Malassezia furfur* with an MIC of 2500 µg/ml. Also, the phytochemical screening gave us an idea about constituents that are responsible for these activities. Happily, our research had formulated and evaluated herbal gel with its ethanolic extract and hence promising alternative for the therapy.

## Materials and Methods [10-24]

### Test Organism

*Malassezia furfur* (MTCC No:1374) was procured from the Microbial Type Culture Collection and Gene Bank, Chandigarh, India.

### Plant Material

Fresh rhizomes of *Zingiber wightianum* Thwaites were collected in the month of August. The plant was identified taxonomically by Dr. Sr.Tessy Joseph, HOD, Department of Botany, Nirmala College, Muvattupuzha.



**Fig 01: Rhizomes of *Zingiber wightianum* Thwaites**

### Preparation of Plant Extract

Ethanol Extraction: Fresh rhizomes of *Zingiber wightianum* Thwaites was rinsed thoroughly in running

tap water, shade dried at room temperature(22-25°C) for a span of 21 days and pulverized into coarse powder using a mechanical grinder. About 17g of powder was weighed and packed inside a filter paper and subjected to Soxhlet extraction using ethanol as a solvent. The extraction was continued until the color of the solvent in the column turns colorless. The solvent was removed by distillation and the resulting semisolid mass was dried. This extract was further subjected to preliminary phytochemical screening in order to detect the chemical constituents present in it.

### Antifungal Activity

Ingredients of nutrient agar media

Sabourauds Dextrose Agar with	
chloramphenicol	- 6.14
Virgin coconut oil	- 0.2
Water	- 100ml
pH (at 25°)	: 5.5±0.2

### Media Preparation and Antifungal Analysis

6.14 g of Sabourauds Dextrose Agar with chloramphenicol was dissolved in 100 ml distilled water to which 0.2ml Virgin coconut oil was added and this was sterilized by autoclaving at 121°C for 15 minutes at 15 lbs pressure. After sterilization, the media was cooled to 45°C in water bath. Then 25ml of media was poured into petri plates that were previously sterilised and allowed to solidify. After that, yeast culture of *Malassezia furfur* with visible turbidity was swabbed on the surface of SDA plates and kept for drying the inoculum. Then, using sterile corkborer of 8 mm diameter, wells were prepared on the agar media, samples with different concentrations were added to the well. Miconazole was kept as control. After the complete diffusion of sample into the agar well, the plates were then incubated at 25°C for 7-10 Days.

### Minimum Inhibitory Concentration

The minimum inhibitory concentration of the ethanolic extract was estimated against *M.furfur* only, which showed better zone of inhibition. The minimum inhibitory concentration was determined by taking various series of concentration. Equal amount of culture medium was added and then yeast culture of *Malassezia furfur* was to be inoculated. Here the standard taken was miconazole. Plates containing cultures were then incubated at 30°C for 7 days. The plate with the least concentration of the extract with no visible growth was

considered as minimum inhibitory concentration of the extract.

#### Analytical Method Used in The Determination of Plant Extract

The use of UV spectrophotometry method was carried out for the analysis of drug using double beam Shimadzu1700 spectrophotometer. Determination of  $\lambda_{max}$  was done by dissolving the extract in little quantity of Ethanol and further diluting it with distilled water. It was then scanned for maximum absorbance in UV double beam spectrophotometer (Shimadzu- 1700) in the range, 200 to 400 nm, using Ethanol-distilled water solution mixture as blank

#### Standard graph for plant extract

A concentration of 1mg/ml was obtained by dissolving 100mg of the plant extract in 10ml of ethanol and making up the remaining volume using water. Series of solutions were prepared with concentration range of 10,20,30,40,50 $\mu$ g/ml by pipetting 1,2,3,4,5 ml from stock solution and then they were made up to 100ml with water. Linearity for detector response was observed in the concentration range of 0-50  $\mu$ g/ml. The absorption maxima method was used to prepare the calibration curve. By appropriate dilution of stock solution and scanning the solutions in the spectrum mode from 200 nm to 400 nm, the  $\lambda_{max}$  at 260 nm was chosen for analysis. The calibration curve was plotted and the concentration of the sample solution was determined.

#### Preparation of Gels

Required amount of (0.5g) extract was dissolved in a solvent mixture containing ethanol and water. Then the required amount of carbopol was weighed and transferred to the solvent mixture. The polymer was then allowed to swell completely without constant stirring. After the solution completely swelled up, it was continuously stirred by a mechanical stirrer for about 2 hours. After 2 hours, triethanolamine was added to obtain a pH (5.7-6.8) similar to that of the skin and to formulate a gel with required consistency.

**Table 01: Formulae used for the development of Gel**

Ingredients (%w/w)	Gels		
	F1	F2	F3
Extract	0.5	0.5	0.5
Carbopol-934	1	1.5	2
Triethanolamine	0.4	0.5	0.6
Ethanol	40	45	50
Water	60	55	50

#### Evaluation of Gel

##### Homogeneity

All developed gels that were set in various containers were tested visually for homogeneity, appearance and presence of aggregates.

##### Grittiness

All the formulations were assessed for the presence of particles, microscopically.

##### Measurement of Ph

A digital pH meter had been used to determine the pH of the various gel formulations this was done by dissolving one gram of gel was in 100 ml of distilled water. The experiment was carried out in triplicate and average values were calculated.

##### Drug content

500 mg of gel was dissolved in 10ml ethanol in a volumetric flask and made up to 100ml with water. This was then kept for a time span of 2 hours in a shaker and shaken well in order to mix it properly. The solution was then further filtered using filter paper and the drug content was measured spectrophotometrically at 260 nm against corresponding gel concentration as blanks. The drug concentration of the gel was then calculated by comparing absorbance of the gel solution to the slope of standard curve of ethanolic extract of *Zingiber wightianum* Thwaites.

Drug Content = (Concentration  $\times$  Dilution Factor  $\times$  Volume taken)  $\times$  Conversion Factor

##### Viscosity study

The measurement of viscosity of the formulated gels were done with the help of a Brookfield Viscometer. The samples to be determined were rotated at 6 rpm using spindle no. 64. At each speed, the corresponding dial reading were noted.

##### Spreadability

It is determined by parallel plate method by pressing 1 g of a sample between two 20 X 20 cm horizontal plates, the upper of which weighed 125 g. The spread diameter  $\Phi$  was measured after 1 min.

#### Result

##### Phytochemical Screening

Ethanolic extract of *Zingiber wightianum* was assessed for the existence of phytochemicals and it exhibited the presence of alkaloids, carbohydrates, tannins, flavonoids, saponins and phenols. These phytochemical compounds identified were of huge medicinal importance and thus possess antifungal activities.

**Table 02: Phytochemical screening**

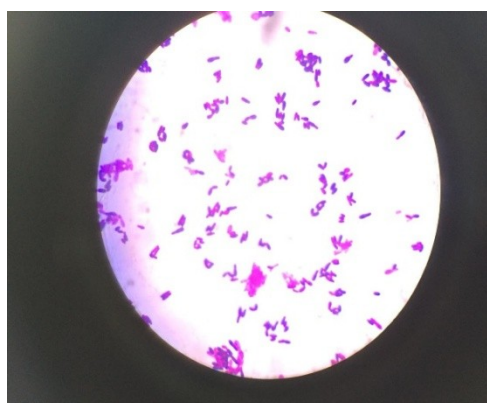
Sl No.	Test	Ethanolic Extract
1	Alkaloids	+
2	Carbohydrates	+
3	Glycosides	-
4	Tannins	+
5	Flavonoids	+
6	Saponin	+
7	Phenols	+
8	Anthocyanin	-
9	Steroids	-
10	Proteins and Amino acid	-

(+) -positive

(-) –negative

**Antifungal Activity**

Ethanolic extract of the rhizomes were found to be effective against the tested pathogen by agar well diffusion method. The ethanolic extract showed 15mm zone diameter in 2500µg/ml which was found to be the minimum inhibitory concentration of the drug. The standard drug miconazole exhibited 34mm in diameter of inhibition in culture medium of *Malassezia furfur*. When comparing the inhibitory activity of the positive control(miconazole) to that of the ethanolic extract, the latter exhibited significantly better inhibitory activity against the fungus. While the negative control ethanol exhibit only little inhibitory activity.



**Fig 01: Microscopical View of *Malassezia furfur* in SDA+ Virgin coconut oil**



**Fig 02 A Ethanolic Extract Against *M.furfur***  
 A:250µg/ml,B:500µg/ml,C:1000µg/ml D:Miconazole (standard) E:Ethanol(control)



**Fig 02B: Ethanolic Extract Against *M.furfur***  
 A:2500µg/ml, B:5000µg/ml, C:10000µg/ml D: Miconazole (standard) E: Ethanol(control)

**Table 03: Zone of Inhibition In *M.Fufur***

Test Result of Samples on <i>Malassezia furfur</i>			
Sl.No	Extract	Concentration	Zone of Inhibition (Diameter in mm)
1	Ethanolic Extract	10000	20.67±0.577
		5000	17.67±0.577
		2500	14.67±0.577
		1000	-
		500	-
		250	-
2	Miconazole	2500	35±1.0

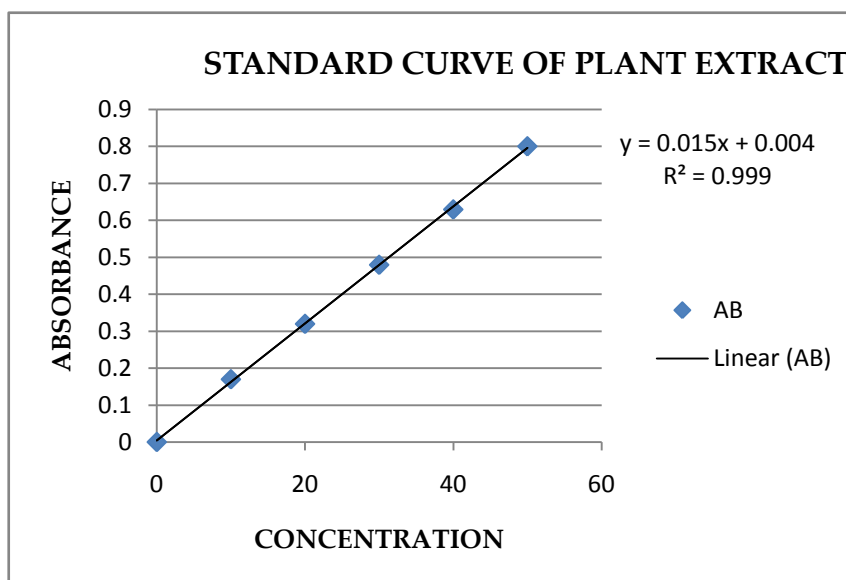
(-) – No zone of Inhibition

### Analytical Method Used in The Determination of Plant Extract

The calibration curve was prepared and the concentration of the sample solution was determined. The result is depicted in the table below. Beer Lambert's law was obeyed in the concentration range of 0-50 µg/ml with regression coefficient of 0.9996

**Table 04: Spectrophotometric data for the estimation of plant extract**

Concentration (µg/mL)	Absorbance
0	0
10	0.170
20	0.320
30	0.480
40	0.630
50	0.800



**Fig 03: Standard curve of Plant Extract**

### Evaluation of Gel

In the current analysis, attempts were made to formulate and evaluate topical gel using ethanolic extract of *Zingiber wightianum*. The prepared gel formulation was yellowish orange in colour with a smooth and homogenous appearance. The pH of the formulation was found to be within the range 6-7 and the addition of Carbopol as the polymer was one of the main reasons to obtain such a range. Spreading area obtained showed that the gel is having good spreadability on its application. The drug content was determined to be between 93%-96% and thus it has good antifungal properties.

**Table 05: Physicochemical characteristics of formulations**

Formulation	Homogeneity	Grittiness	Color
F1	+++	-	Yellowish orange
F2	+++	-	Yellowish orange
F3	+++	-	Yellowish orange

Excellent +++, Good ++, Satisfactory +, No grittiness -

**Table 06: pH, Viscosity, Drug content and Spreading Area of different formulations**

Formulation Code	Ph	Viscosity	Drug Content (%)	Spreading Area
F1	6.12	49900	96	2122.64
F2	6.47	51721	95	2041.7
F3	6.52	56680	93	1962.5

## Discussion

### Phytochemical screening

Phytochemical composition of ethanolic extract of *Zingiber wightianum* on mycotoxigenic fungi were evaluated in this project. This screening showed the existence of alkaloids, phenols, flavonoids and carbohydrates. Ayodele *et al* [33] explained in their article about phytochemistry and antifungal activity of *Zingiber officinale*. Active constituents present in ginger extract includes glycosides, alkaloids, tannins, saponins, terpenoids and phenols of which flavonoids, saponins and alkaloids were detected in rich fractions and which leads to high potential for antifungal activity. In agreement with this article phytoconstituents in *Zingiber wightianum* is almost similar and thus it can be just as well concluded that it also possess antifungal activity.

### Antifungal evaluation

Ethanolic extract of the rhizomes were found to be effective against the test organism by agar well diffusion method. The ethanolic extract showed 15mm zone diameter in 2500µg/ml which was found to be the minimum inhibitory concentration of the drug. Kader *et al* [35] explained about the potent antibacterial and antifungal phytochemicals present in ethanol extract of *Zingiber zerumbet* which correlated with our chemical investigations. Crude extracts of *Dittrichia viscosa* were studied for antifungal activity against *Malassezia furfur* and revealed the inhibitory effects of phenolic compounds present in it [24]. Inhibitory effect of natural phenolic compounds on fungal growth were investigated and hence proved that with high conc. of phenolic compounds, there would be remarkable increase in lag phase and decrease in growth rate. This finding could be compared with their molecular structure: a non-polar part to make possible their passage through the membrane and a hydroxyl group

combined with a system of delocalised electrons that confers an acidic character to the molecules, leading to the destabilisation of the cell membrane [25].

### Formulation development

In this study, gel was formulated by using Carbopol 934, alcohol, water and triethanolamine. A total number of three formulations were made by changing the quantity of polymer, triethanolamine, alcohol and water. Basha *et al* [27] used Carbopol 934 as gelling agent. The release of the drug from the formulation was inversely proportional to concentration of Carbopol. Alcohol particularly ethanol was incorporated to dissolve the extract because it is insoluble in water also it is a penetration enhancer. Triethanolamine, a pH balancer and is added dropwise during constant stirring as it provided the required consistency, Water acts as a vehicle.

### Evaluation of formulation

The prepared gel formulation was yellowish orange in colour with a smooth and homogenous appearance.

### Spreadability

Another important physico-chemical parameter is Spreadability as it shows the behaviour of gel that comes out from the tube. Spreadability increases as the viscosity decreases and vice versa. it can be concluded from the data that F1 formulation have better spreading properties as compared with others.

### Viscosity

Patel *et al* [30] reported that viscosity is negatively related to the release of drug from formulation and its permeation through diffusion barriers. It can be seen that when compared to F2 and F3, F1 formulation is less viscous and therefore exhibits better drug release.

pH value of the various gel formulations was observed to be ranging from 6.12-6.52. This is due to the existence of alkaline triethanolamine in the formulation along with acidic Carbopol. This proves the fact that it will not create any skin irritation in individuals.

### Drug Content

Debnath *et al* investigated the evaluation of aceclofenac gel and the drug content was calculated from the calibration curve. This work summed up that the gel containing maximum drug exhibit high efficiency. In our present investigation, formulation F1, F2, F3 contain 96%, 95% and 93% respectively. Among these three formulations, F1 found to have comparatively high drug content and thus more effective.

From the above research, it is evident that Formulation F1 have comparatively good spreadability, less viscous, acceptable pH and high drug content. Thus, it can be

concluded that formulation F1 is the best formulation among three of them and have good drug release and limited skin irritancy [29-32].

### Conclusion

The objective of this project was to look further into the antifungal aspects of *Zingiber wightianum* and to develop a novel phytotherapy for Tinea versicolor. To achieve this goal, the research focused on phytochemical and antifungal evaluation of *Zingiber wightianum* rhizomic extract. From the investigations, ethanolic extract proved its antifungal potential to treat Tinea versicolor against *Malassezia furfur* with an MIC of 2500 µg/ml. Also, the phytochemical screening gave us an idea about constituents that are accountable for these activities. Happily, our research had formulated and evaluated herbal gel with its ethanolic extract and hence promising alternative for the therapy.

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