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Research Article

Antimycotic activity and in-silico validation of bioactive compounds of Roccella montagnei Bél. against clinically isolated onychomycosis fungus Trichophyton mentagrophytes (C.P. Robin) Sabour

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ABSTRACT

Dermatophytes are the most common slow growing contagious agents of cutaneous mycosis and persist as a significant public health issues despite of the availability of a number of antifungal medications. *Trichophyton mentagrophytes* (C.P. Robin) Sabour. is one of the fungal species which influences the dermatophytic diseases. A wide range of clinical dermatophytosis could infect scalp, face, hand, fingernails, toenails, feet, and also produce jock itch and ring worm of the body. Generally, terbinafine, is used to treat dermatophytosis induced by *Trichophyton* species. In most of the cases, synthetic anti-mycotic drugs fail over time resulting in development of resistant strains. Sustainable use of herbal sources has been recognised as novel therapeutic drugs to treat dermatophytic disorders. However, few reports are available on antimycotic activity of lichen species. Therefore, in this study, lichen species such as *Roccella montagnei Bél* was collected from prime locality of Bhubaneswar, Odisha. Antifungal potency of ethanol and methanol extractives of *R. montagnei* was screened by disc diffusion method against the clinical isolates of *T. Mentagrophytes* and standard deviation and error was calculated. The overall study resulted with a positive output and validated through in-silico database. Hence the entire study is based on biomedical evaluation and sustainable use of natural plant supplements for the benefit of human society.

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INTRODUCTION

Dermatophytosis is the epidermal infection of living organisms with direct or indirect contact with harmful microorganisms. It is very common and harmful, to all categories of life. With time, these ignored infections affect internal body parts in immunocompromised patients [1]. The rate of dermatic treatment by synthetic pharmaceuticals is higher than by natural medicinal sources, although it is time-consuming and bears harmful side effects with long-term use. Some well-known derivatives, such as luliconazole, terbinafine, and ketoconazole,are becoming resistant to mutant variants [2, 3]. Hence, the aim here is to localize the sustainable use of lichen species in dermatophytic infections as an effective source without any side effects [4]. In general, lichens are well known for monitoring pollution; here, the involvement of a fruticose lichen species, *Roccella montagnei Bél* [5]. is focused on treating clinically isolated onychomycosis fungus *Trichophyton mentagrophytes* (C.P. Robin) Sabour [6]. The antimicrobial activity confirmed the presence of some potential phytoconstituents in both methanolic and ethanolic extracts of *R. Montagnei. Insilico* docking was also performed by using PubChem and Uniprot, to relate the experimental work [7].

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MATERIALS AND METHODS

Species Collection

In this study, the lichen species was collected from the shoot of *Roystonea regia* (Kunth) O. F. Cook, and identified as *Roccella montagnei Bél*. accession no. 52479 by Komal Kumar Ingle, National Botanical Research Institute (CSIR-NBRI), Lucknow, India. However, we have collected the organism *T. mentagrophytes* from KIIMS Hospital, Bhubaneswar, Odisha. A voucher specimen was deposited at Dept. of Botany, CUTM, Bhubaneswar.

Plant Sample Extraction

Exactly 100 grams of the unrefined lichen powder was extracted with methanol and ethanol (4 times 100 ml each) separately on a flask shaker for 24 to 48 hours, filtered, and concentrated on a rotary evaporator to extract the crude compounds.

Phytochemical Evaluation

Prilimnary phytochemical analyses was done for both methanolic and ethanolic extracts to detect the presence of different class of compounds such as alkaloids, saponins, carbohydrates, steroids, terpenoids, lipids, tannins, phytosterols and flavonoids [8].

Determination of in-vitro Antimicrobial Activity

The methanolic and ethanolic extract was considered for in-vitro antimicrobial assay against *T. mentagrophytes* to observe the effectiveness of *R. montagnei* for the cure of dermatophytosis. Sterilised discs (Himedia, SD067-1VL, 6mm sterile Susceptibility test disc) were dipped in 10 microliter of each extracts separately and allowed to dry for evaluation of antifungal activities by disc diffusion methods in separate petri plates containing standardized media combined with Sabouraud's dextrose agar (SDA) and Potato Dextrose agar (PDA) in 2:1 ratio. As the fungus was unable to grow in the existing media, therefore, standardisation of media was done for the growth of the clinical isolates in *in-vitro* condition. To measure the effectiveness, respective organic solvents are taken as negative control and the usual drug terbinafine was taken as a positive control. The plates were observed up to 5 to 8 days at 34-37 °C inside a culture incubator and after one week, the zone of inhibition was measured by using zonic scale. The performed in-vitro work was verified through in-silico validation.

In-Silico Validation

Retrieval of phytoconstituents

In this study, 26 phyto-constituents of *R. montagnei* were selected by searching their information in various published literature [9, 10,11,12]. The physio-chemical record of these bioactive compounds was obtained through the database PubChem [13] in structure data format (SDF). Employing the BIOVIA Discovery Studio 4.5 Visualizer, the 3D structures were transformed to PDB format [14].

Lipinski rule of five

Lipinski's Rule of Five [15], is the necessary requirement to choose a potential drug candidate to find out an orally active medicine. The drug molecule has to adhere the specifications of parameters like molecular mass should be <=500 Dalton, logP value must be <=5, the no. of H- bond donor should be <=5, the no. of H- bond acceptors should be <=10, and molar refractivity value must be within 40-130. A molecule fails to qualify as a drug if its conditions are violated. TargetNet web server [16] employed to conjecture Lipinski's Rule of Five.

Validation of toxicity

The phyto-compounds were classified implementing Lipinski's Rule of Five. Potential compounds were vetted with the mcule database [17] to check the toxicity results of the compounds for their drug-likeliness properties.

The protein sequence and structural information, of target genes of dermatophytosis viz., CHI3L2, MEP3 and DPPV are retrived from the UniProt database [18]. All the experimental structures of the target proteins of dermatophytosis caused by *T. mentagrophytes* are accessed through the Protein Data Bank (PDB) database [14]. Chain A of each structure chosen for analysis.

Specific target proteins are retrived using Uniprot tools [18], and three phyto-compounds; orcinol, 4-hydroxy benzoic acid, and divarinol, were marked efficient for in-silico drug docking among 26 compounds after considering remarkable parameters with the help of PubChem database [13].

RESULTS AND DISCUSSIONS

The antimicrobial activity of both methanolic and ethanolic extracts of *R. montagnei* shows effective results against *T. mentagrophytes* with an identified zone of inhibition. Standard deviation of three observations was depicted in Table 1. Standard error graph was plotted and shown in Figure 1.

Table 1. Antifungal activity of lichen extracts against *T. Mentagrophytes* compared with +ve and -ve controls

Solvents	*Zone of Inhibition (mm)
Methanol	13.67 ± 0.89
Ethanol	11± 0.58
Terbinafine	3.33± 0.33
Control	0

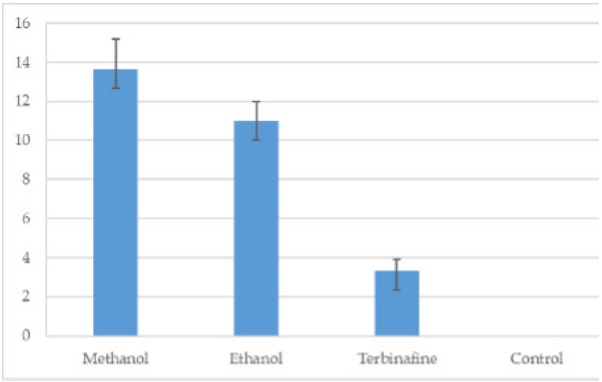

Figure 1. Zone of inhibition of lichen extracts against *T. Mentagrophytes*

Maximum zone of inhibition was recorded as 13.67 ± 0.89 mm for methanolic extract followed by ethanolic extract with 11±0.58 mm. Terbinafine showed minimum ZoI of 3.33± 0.33 mm, suggesting that the methanolic extract is effective than ethaloic extract and Terbinafine (Figure 2).

Prelimnary phytochemical screening shows the presence of six class of phytocompounds in methanol and seven class of phytocompounds in ethanol extracts respectively from the extracts of *R. montagnei*, which validates the possible positive effect of antifungal results (Table 2).

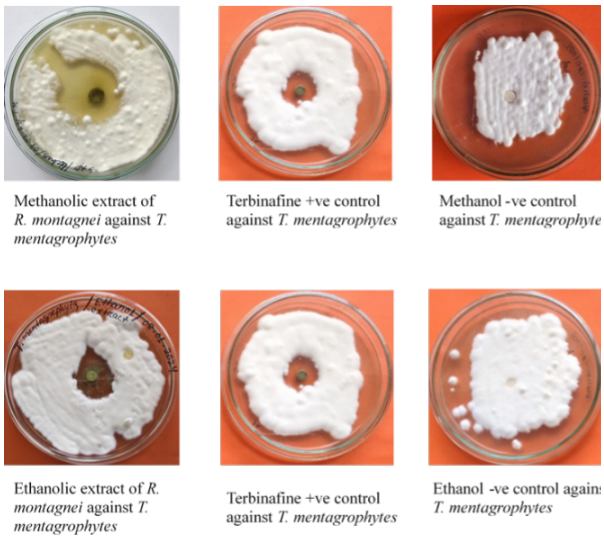

Figure 2. Zone of inhibition of lichen extracts against T. *Mentagrophytes* by disc diffusion method

Twenty-six phytocompounds have been retrived from *R. Montagnei* from the reported literature, out of which only three follow Lipinsky's rule of five and are non-toxic in nature. The targeted three proteins such as chitinase-3-like protein 2, extracellular metalloproteinase 3 (Fungalysin), and dipeptidyl-peptidase 5 (alpha-keratin) are responsible for dermatophytic infections [10, 11]. The details of proteins available in Table 3 and Protein Data Base (PDB) ID taken for one protein, 4P8V, and the two are modeled using the Swiss Modeler tool.

Phyto-constituents from *R. montagnei*

Twenty-six phyto-constituents from the thallus of *R. montagnei* has been selected for this study and information was depicted in Table 3.

Selection of Non-toxic Phyto-constituents Following Lipinski Rule of Five Having Drug Likeliness Properties

TargetNet's web server was used to apply the Lipinski rule of five, which eliminated five compounds out of 26 retrieved phytoconstituents. Out of the 21 phytoconstituents, only three compounds fulfilling Lipinski's rule of five and are found non-toxic in nature with drug-likeliness properties (Table 4).

Table 2. Phytochemical screening of methanolic and ethanolic extracts of *R. montagnei*

Test	Methanol Extract	Ethanol Extract
Alkaloids	+++	++
Saponins	-	-
Carbohydrates	-	++
Steroids	+++	+++
Terpenoids	+	+++
Lipids	-	-
Tannin	+++	+++
Phytosterols	+	+++
Flavonoids	+++	+++

Table 3. Retrieved phytoconstituents of *R. montagnei*

Sl. No.	Chemical Name	Chemical formula	PubChem ID
1	Methyl-γ-Orsellinate	C9H10O4	76658
2	Roccellatol	C12H16O7	139591667
3	Ergosta-5, 7, 22-triene-3β-ol	C28H44O	21139765
4	Ergosta- 7, 22-diene-3, 5,6-triol	C28H46O3	10181133
5	Ethyl 2,4-dihydroxy-6-methyl-3-(oxolan-2-yl)benzoate	C14H18O5	142736239
6	Erythrin	C20H22O10	72946996
7	Lecanoric acid	C16H14O7	99613
8	Rocellic acid	C17H32O4	11449446
9	Orcinol	C7H8O2	10436
10	Erythritol	C4H10O4	222285
11	Beta sitosterol	C29H50O	222284
12	Gallic acid	C7H6O5	370
13	Syringic acid	C9H10O5	10742
14	P-coumaric acid	C9H8O3	637542
15	Caffeic acid	C9H8O4	689043
16	Ferulic acid	C10H10O4	445858
17	Chlorogenic acid	C16H18O9	1794427
18	4-hydroxy benzoic acid	C7H6O3	135
19	Ethyl divaricatinate	C13H18O4	179627
20	Divarinol	C9H12O2	3083600
21	Atranol	C8H8O3	458186
22	Ethyl haematommate	C11H12O5	3940691
23	Ethyl orsellinate	C10H12O4	75653
24	Sekikaic acid	C22H26O8	12315460
25	Ar-Turmerone	C15H20O	160512
26	Lichenxanthone	C16H14O5	5358904

Table 4. Lipinski rule of five analyses of the phytoconstituents of *R. montagnei*

Sl. No.	Chemical Name	Chemical formula	PubChem ID
1	Methyl-γ-Orsellinate	100.0%	Toxic
2	Roccellatol	100.0%	Toxic
3	Ethyl 2,4-dihydroxy-6-methyl-3-(oxolan-2-yl)benzoate	100.0%	Toxic
4	Lecanoric acid	100.0%	Toxic
5	Rocellic acid	100.0%	Toxic
6	Orcinol	100.0%	Non-toxic
7	Erythritol	100.0%	Toxic
8	Gallic acid	100.0%	Toxic
9	Syringic acid	100.0%	Toxic
10	P-coumaric acid	100.0%	Toxic
11	Caffeic acid	100.0%	Toxic
12	Ferulic acid	100.0%	Toxic
13	4-hydroxy benzoic acid	100.0%	Non-toxic
14	Ethyl divaricatinate	100.0%	Toxic
15	Divarinol	100.0%	Non-toxic
16	Atranol	100.0%	Toxic
17	Ethyl haematommate	100.0%	Toxic
18	Ethyl orsellinate	100.0%	Toxic
19	Sekikaic acid	100.0%	Toxic
20	Ar-Turmerone	100.0%	Toxic
21	Lichenxanthone	100.0%	Toxic

Molecular Docking

For dermatophytosis caused by *T. mentagrophytes*, three proteins viz., Chitinase-3-like protein 2 (CHI3L2), Extracellularmetalloproteinase 3 (Fungalysin) (MEP3) and Dipeptidyl-peptidase 5 (alpha-keratin) (DPPV) were selected (Table 5) from the reported literature for molecular docking using CB DOCK 2.

The selected 3 phytochemicals were docked against the 3 selected protein targets by using CB DOCK 2, where divarinol scored the highest binding affinities of -6.3 kcal/mol, -6.0 kcal/mol, and -6.2 kcal/mol (Table 6, Figure 3).

Table 5. Selected target proteins of *T. mentagrophytes*

Entry ID (Uniport)	Protein Name	Gene Name	Aminoacid Length
Q15782	Chitinase-3-like protein 2	CHI3L2	390 4P8V (1.64)
Q6WIH8	Extracellularmetalloproteinase 3 (Fungalysin)	MEP3	633 modelling
Q9UW98	Dipeptidyl-peptidase 5 (alpha-keratin)	DPPV	726 modelling

Table 6. Molecular docking study of selective bioactive compounds from R montagnei

Protein	Docking score (ΔG, kcal/Mol) Reported compound		
	Orcinol	4-hydroxy benzoic acid	Divarinol
Chitinase-3-like protein 2	-5.6	-5.0	-6.3
Extracellular metalloproteinase3 (fungalysin)	-5.6	-5.9	-6.0
Dipeptidyl-peptidase5 (alpha-keratin)	-5.2	-5.8	-6.2

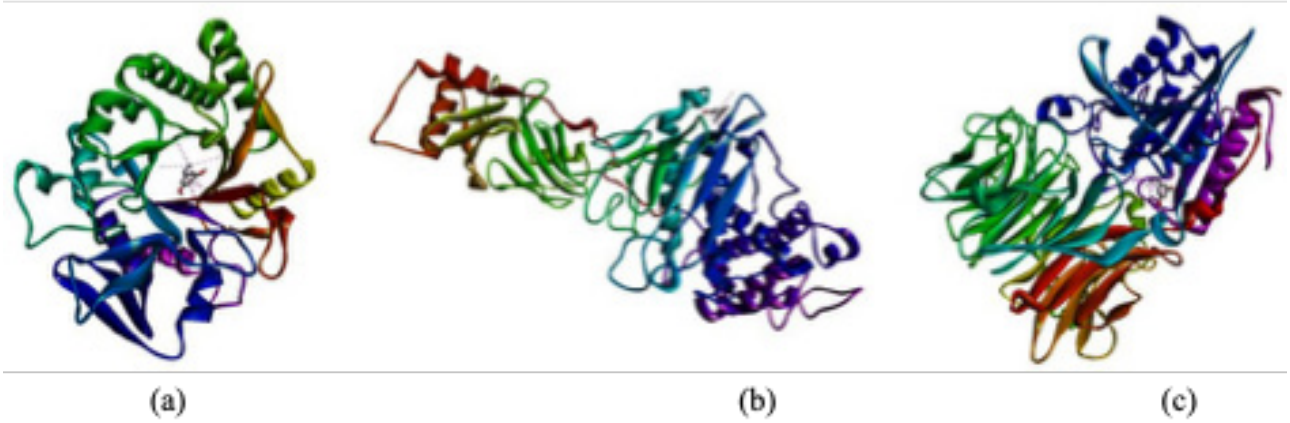

Figure 3. 3D Interaction of target proteins with phytocompounds [*(a) chitinaseprotein-divarinol; (b) fungalysin-divarinol; (c) alpha-keratin-divarinol]

Lichens are rarely used drug derivatives in natural medicinal system, although they are so efficient as nutritional sources for mankind. The presence of proficient phyto-constituents reported from *R. montagnei*, are capable of treating dermatophytosis caused by *T. metagrophytes*. This study revealed that the ethanolic and methanolic extracts of *R. montagnei* showed in vitro antifungal activity against onychomycosis caused by *T. mentagrophytes* and inhibit the growth of pathogen in a shorter period of time. Disk diffusion technique is considered in this study because it is one of the much simpler experiments for antifungal susceptibility test for dermatophytes and also easy to perform in the regular clinical practices as the broth dilution method [19]. Ethanolic extract shows more active compounds rather than the methanolic extract. In-silico docking of three refined phytocompounds from *R. montagnei* against three target proteins also showed effective results but amongst these three compounds divarinol is proved to be a potent phyto-constituent which scored with the highest binding affinities of -6.3 kcal/mol, -6.0 kcal/mol, and -6.2 kcal/mol and further research can be carried out to isolate this compound as a source for novel drug discovery. An investigation has [20] reported that methanol extracts of lichen species Proto usnea poeppigii inhibits a few fungal pathogens including *T. mentagrophytes* because of the presence of different metabolites such as usnic acid, isodivaricatic acid and divaricatinic acid which shows that lichen species can be a source for dermatophytic pathogens. This study could be used in discovering effective drugs against *T. mentagrophytes* [21].

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CONCLUSION

Ethanolic and methanolic extract of *R. montagnei* showed inhibiting zones against *T. mentagrophytes*. In-silico docking also revealed the antidermatophytic effect of three phytoconstituents such as orcinol, 4-hydroxy benzoic acid and divarinol. Out of these three, divarinol is found to be more effective with highest binding affinities with the selected three proteins. The overall work represents that R. montagnei can play a vital role as a source of natural medicines aginst *T. mentagrophytes.*

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

USE OF AI FOR WRITING ASSISTANCE

Not declared.

ETHICS

There are no ethical issues with the publication of this manuscript.

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