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Pharmacognostical, Phytochemical and Antifungal Analysis of "*Tabernaemontana alternifolia*" Leaf Extract and Formulation of its Nail Lacquer

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Abstract: *Tabernaemontana alternifolia*, also known as kundalam paalai, belongs to the family Apocynaceae. Leaf has antibacterial, anthelmintic, antioxidant and healing properties against the conditions of the nervous system ,liver ,kidney ,skin. It is also used to treat diabetes, chronic bronchitis, cardiac problems and snake bites . Phytochemical screening showed the presence of alkaloids, phytosterols , triterpenoids, proteins, phenolic compounds, tannins, quinones, flavonoids, glycosides. The purpose of current study was to access the plant extract by agar well diffusion method and formulation of its nail lacquer and to evaluate the same.

Keywords*: Tabernaemontana alternifolia, Anti Fungal Activity, Nail Lacquer.*

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I. INTRODUCTION

Kundalam Paalai, also known as *Tabernaemontana alternifolia L.* (Apocynaceae), is a highly prized medicinal plant in traditional folk medicine. Numerous investigations have been conducted to evaluate the pharmacological potential of the plant's various sections, including the roots, stem, flower, bark, and leaves[1]. The plant is recognised ethnobotanically for its antibacterial, anthelmintic, antioxidant, and healing qualities against conditions of the nervous system, liver, kidneys, skin, respiratory system, and eyes.[2] *Tabernaemontana alternifolia*, a flowering shrub or small to medium-sized tree commonly found in savannahs, rocky areas, and forest understories, is traditionally used to treat various conditions including sexual diseases, diabetes, chronic bronchitis, heart problems, and snakebites. Plants in this genus are characterized by tubular white flowers, follicular fruits with seeds enclosed in a yellow to reddish aril, and a milky or watery latex, earning them the common name "milkweed." This latex is often associated with their biological activity.[3] Studies have demonstrated the antifungal properties of different leaf extracts of T. alternifolia against fungal strains such as *Rhizopus mucor* , *Candida albicans, Aspergillus niger, Gibberella fujikori, Penicillium chrysogenum*, and *Aspergillus terreus*, using the agar well plate method. *Trichoderma viridins and Rhizopus mucor* both exhibited a minimum and maximum antifungal effect. Of the seven fungi that were analysed, the growth of *A. terreus and C. albicans* was found to be considerably inhibited, whilst that of *R. mucor, P. chrysogenum*, and *A. niger* was found to be significantly suppressed.[4] Ethanol was significantly more effective than other solvents at extracting antimicrobial compounds, according to the inhibition zone values.[5]

II. METHODOLOGY

- *Collection of Plant Material*

Mature leaves of *Tabernaemontana alternifoila* were freshly gathered on November 2025,near kadukkaserry, shornur located in the Palakkad district. The plant material was authenticated by Dr. P.S. Udayan ,a botanist from the Department of Botany at Sree Krishna College, Guruvayur and Herbarium has been deposited at pharmacognosy department ,Nehru college of pharmacy, Thrissur ,for future reference. *Tabernaemontana alternifolia* leaves were

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separated from twigs and shade dried for about 10-15 days, powdered with mechanical grinder and stored in an air tight container for further studies.

➢ *Extraction*

Dried leaves of *Tabernaemontana alternifolia* were extracted with ethanol using maceration.

➢ *Pharmacognostical Studies*

- *Macroscopic Characteristics*

The macroscopy of fresh leaves were studied according to standard methods. Sense organ can be used to test crude drug. This is colour, same smell, size and shape other handle tester and the types of leaves depending on margin apex, base surface type and venation. The macroscopic study is there for a macro anatomy of the various components of the plant that can easily be seen by the use of naked eye or a magnifying lens.[6]

- *Microscopic Characteristics*

For microscopy hand section of leaf and stem was taken, stained and mounted following usual micro techniques. For transverse section leaf and stem was taken, stained by using phloroglucinol.HCL and mounted in glycerin. For powder microscopy the whole plant powder was taken, stained by using sudan red and mounted in glycerin.[7]

➢ *Physico-Chemical Analysis*

- *Determination of Total Ash*

In an ignited and tared crucible, 2g of ground dried material was accurately weighed (usually of platinum and silica). The material was spread in an even layer and ignited by gradually raising the temperature to 500-600°c until it was white, indicating the absence of carbon. Then it was allowed to cool in desiccators before being weighed.[8]

- *Determination of Water-Soluble Ash*

The ash was obtained as in above total ash, heated for 5 minutes with 25°c of water, and filtered. Insoluble debris was collected on ash-free filter paper, rinsed with hot water, and ignited at 450°c for 15 minutes. After that, the ash was weighed.[8]

- *Determination of Acid-Insoluble Ash*

The ash was obtained as in above total ash, the ash boiled for 5 minutes with 25 mL of 2M HCL acid, filtered and the insoluble matter was collected on ash-free filter paper. It was ignited, cooled in a desiccator. [8]

- *Determination of Sulphated Ash*

The residue was moistened with 1mL sulphuric acid and gently heated till white fumes no longer evolved. After igniting at 800 ± 25°C, all back particles disappeared. The crucible was allowed to cool and a few drops of sulphuric acid were added and the crucible was heated. After igniting it again, the crucible was allowed to cool and was weighed.[8]

- *Determination of Extractive Value*

The powdered material of the drug (1 g) was subjected to extraction with different solvents like water, acetone, chloroform, benzene, N-hexane, petroleum ether, ethyl acetate, ethanol. 25 mL of filtrate was evaporated to dryness in a tarred bottom china dish. It was then dried at 105°C and weighed.[9]

- *Determination of Loss on Drying*

In a Petri dish, 1 gm of powder was accurately weighed and kept in a hot-air oven at 105°C for four hours. After cooling in a desiccator, the weight loss was measured. This procedure was repeated several times until the weight remained constant.[10]

- *Determination of Swelling Index*

1g of plant material into 25ml measuring cylinder and add 25ml of water. Shake the mixture thoroughly every 10 min for 1 hour allow to stand for 3 hours at room temperature.Measure the volume in ml occupied by the plant material,including any sticky mucilage.[10]

- *Determination of Foaming Index*

1g of plant material and add 100ml of water in a conical flask. Cool and filter into a 100ml volumetric flask. Pour the decoction into 10 stoppered test tubes. Dilute to 10ml with water. Allow to stand for 15min. Measure the height of the foam.[10]

➢ *Preliminary Phytochemical Screening*

Phytochemical screening conducted as per WHO guidelines.[11]

- *In- Vitro Antifungal Test-Agar well Diffusion Method*

The test organism used is candida albicans (ATCC 10231), standard drug was fluconazole (50µg).Inoculum was prepared by using stock culture were maintained at 4°C on sabouraud dextrose agar slant. The stock cultures were subcultured to an agar plate containing Sabouraud dextrose broth medium and incubated at room temperature for 48 hours to obtain active cultures. The antifungal activity of the plant extract was studied by using Sabouraud dextrose agar medium and the agar well diffusion method. The petri dish was filled with medium. Once the medium was set, a sterile swab soaked in fungal solution was used to spread the inoculums over the solid medium. Ethanol was used as the control, and fluconazole was used as the standard. The sterile wells of the SDA medium were filled with fluconazole, ethanol, plant extract, and nail lacquer. The medium was incubated in an incubator at 37°C for 48 hours. The diameter of the zone of inhibition was measured to study the antifungal activity. [12]

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➢ *Formulation of Nail Lacquer*

Table 1 Formulation of Nail Lacquer

SL. NO	INGREDIENTS	QUANTITY
1	Tabernaemontana alternifoila leaf extract	5ml
2	Nitrocellulose	10 ml
3	Ethyl acetate	40ml
4	Dibutyl phthalate	5ml
5	Acetone	30 ml
6	Ethyl cellulose	5ml

➢ *Procedure*

Required quantity of nitrocellulose was taken into the beaker and to that required amount of ethyl cellulose was added and then both mixtures were dissolved in ethyl acetate. Then the following ingredients are weighed accurately 5ml of dibutyl phthalate, 30ml of acetone, and some alcohol were added to the above mixture. The afore mentioned combination was continually swirled at 100 rpm in a magnetic stirrer. After that the herbal extract of *Tabernaemontana alternifolia* leaf extract 1ml was added to the above solution. The above mixture was mixed continuously until a clear solution was formed.[13]

➢ *Evaluation Test for Nail Lacquer*

- *Appearance and consistency*

Pour a small amount of the nail lacquer onto a glass plate. Visually inspect the flow and look for any gritty particles, discoloration, or phase separation. examine the smooth, uniform flow without visible particles or separation.

- *Drying Time*

Apply a thin layer of the nail lacquer to a clean glass slide. Start a stop watch immediately. At regular intervals, gently touch the film with a finger. Record the time it takes for the film to be dry to touch.

- *Water Resistance*

Apply a continuous film of the lacquer to a pre-weighed glass plate. weigh the plate with the dried film. Submerge the plate in water for a specified period. Remove the plate, allow excess water to drip off, and reweigh it. A smaller increase in weight indicates better water resistance.

- *Peel Adhesion*

Apply the lacquer to a nail and allow it to dry completely. Attempt to peel off the film from the nail plate using a finger nail or cuticle pusher. assess how easily the film peel of and if it comes off in a single, continues layer.

- *Viscosity*

Ensure the viscometer is caliberated, maintain the sample at consistent room temperature, lower a specific spindle in to the lacquer until it is immersed to the marked line on the shaft. record the stabilised viscosity value in centipoise.

- *pH*

A digital pH meter is used to measure the acidity or alkalinity of the nail lacquer, often diluted with acetone or ethanol.[13]

III. RESULTS

➢ *Collection and Authentication of Leaves of Tabernaemontana alternifoila*

The leaves of *Tabernaemontana alternifoila* were collected and authenticated. These leaves were subjected to further evaluation.

Fig 1 Leaves of *Tabernaemontana alternifoila*

➢ *Extraction*

Percentage yield of solvent extract=10.5%W/W

➢ *Pharmacognostical Studies*

- Macroscopic Characteristics

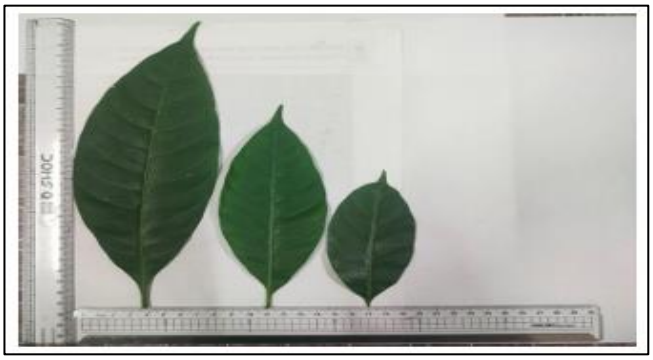

Fig 2 Macroscopic Characteristics of *Tabernaemontana alternifolia*

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Table 2 Macroscopic Characteristics of *Tabernaemontana alternifolia*

Sl No	Features	Observation
1	Colour	Green
2	Size	Leaf :13-27cm long, 5-7cm wide
3	Shape	Elliptical
4	Odour	Slight or faint odour
5	Petiole	Flattened arch
6	Margin	Entire wavy
7	Base	Swollen or slightly clasping

➢ *Microscopic Characteristics*

- *Transverse Section*

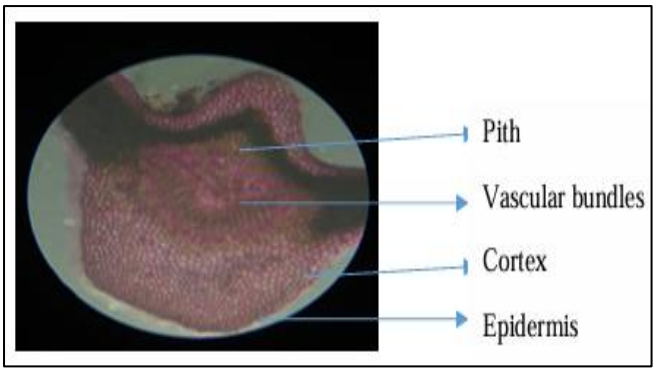

Fig 3 Transverse Section of *Tabernaemontana alternifolia*

➢ *Powder Microscopy*

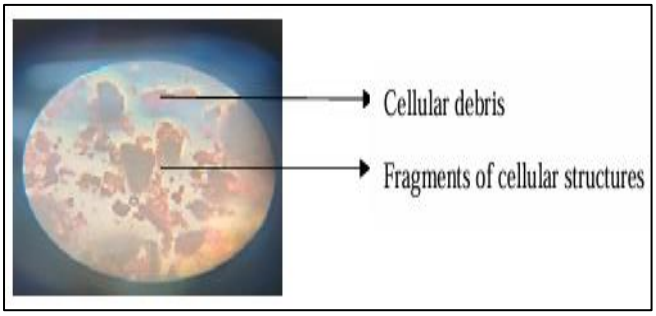

Fig 4 Powder Microscopy of *Tabernaemontana alternifolia*

➢ *Stomatal Index*

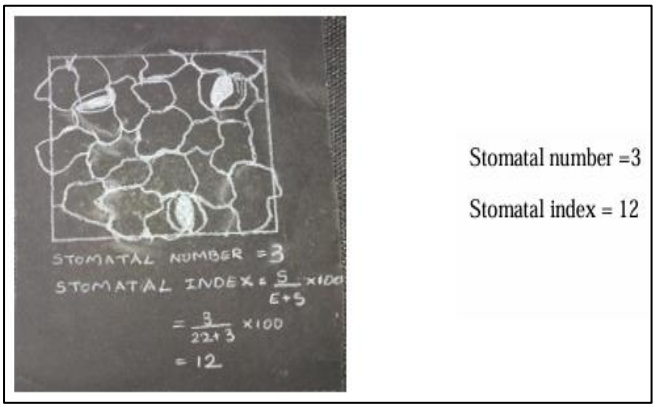

Fig 5 Stomatal Index of *Tabernaemontana alternifolia*

➢ *Vein Islet Number and Vein Termination Number*

Fig 6 Vein Islet and Vein Termination Number of *Tabernaemontana alternifolia*

➢ *Physico-Chemical Analysis*

Table 3 Physico Chemical Analysis

Parameter	Result (%w/w)
Total ash	11
Acid insoluble ash	1.63
Water soluble ash	5
Water soluble extractive value	7
acetone	6
Ethyl acetate	1
Ethanol	10.5
n-hexane	4
chloroform	2
Benzene	3
Petroleum ether	3
Moisture content	3.4
Foaming index	0
Swelling index	1

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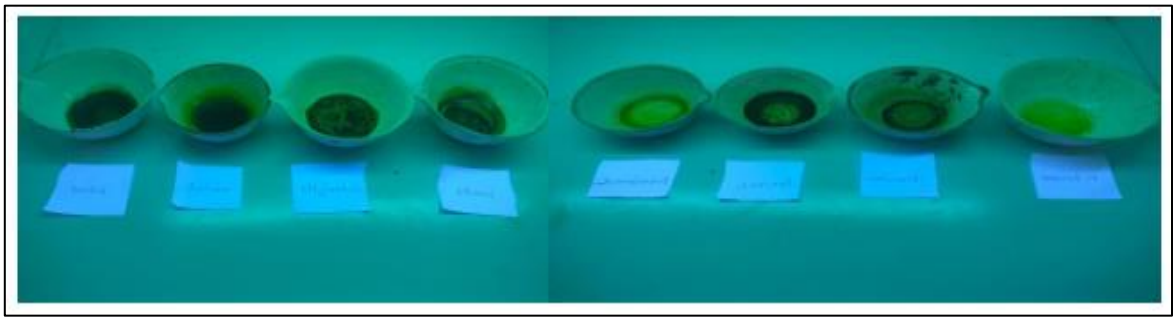

Fig 7 Different Extracts in UV Chamber

Table 4 Colours of Extract in Day Light and Placed in UV Chamber

Solvents	Colour of extract	Colour of extract placed in UV chamber
Water	Deep coffee brown	Dark green
Acetone	Olive green	Dark green
Ethyl acetate	Dark olive green	Dark green
Ethanol	Coffee brown	Dark green
N- hexane	Bright golden yellow	Bright green
Chloroform	Olive green	Dark green
Benzene	Dark green	Dark green
Petroleum ether	Mustard yellow	Bright green

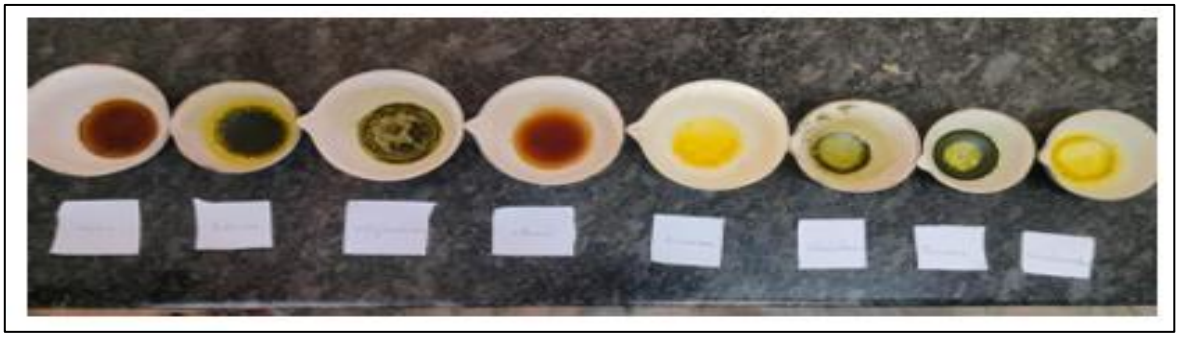

Fig 8 Different Extracts in Day Light

- *In Vitro Antifungal Activity Test for Tabernaemontana Alternifoila Extract*

- *Agar well Diffusion Method*

Table 5 Zone of Inhibition of Leaf Extract and Nail Lacquer.

Name of microorganism	Zone of inhibition (mm)			
	Standard Fluconazole(50µl)	Negative Control	T1 (400µl)	T2 (400µl)
Candida albicans	**20mm**	**-**	**18 mm**	**15mm**

Fig 9 Zone of Inhibition of Leaf Extract and Nail Lacquer.

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➢ *Formulation of Nail Lacquer*

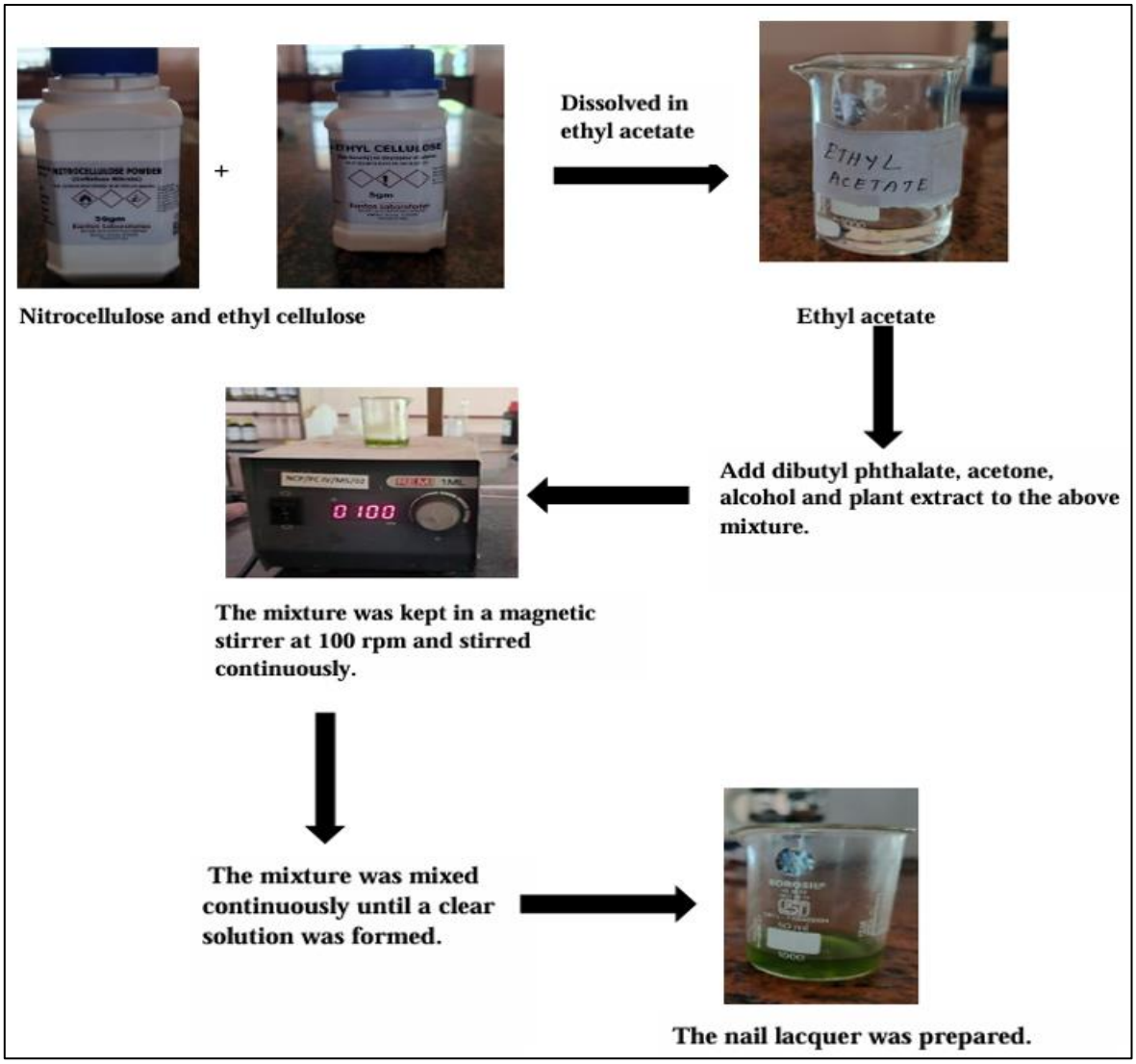

Fig 10 Formulation of Nail Lacquer

➢ *Evaluation Test for Nail Lacquer*

- Appearence and consistency : The goal is smooth, uniform flow without visible particles.
- Drying time : Record the time it takes for the film to be dry to touch .The drying time of nail lacquer was found to be 1min 30 seconds.
- Water resistance : A smaller increase in weight indicates better water resistance

✓ Initial weight : 5.28g (weight of the plate with dried film)
✓ Final weight : 5.28g (submerge the plate in water for 24hours then removed and re-weighed)

- pH :The pH of the nail lacquer was found to be in the range of 4.40, which is considered suitable for application to the nail plate without causing irritation.
- Peel adhesion: Assess how easily the film peels off and if it comes off in a single, continuous layer.
- Viscosity : viscosity of nail lacquer was determined by using brook field and was found to be 341.9 centipoise.

IV. CONCLUSION

The plant was analyzed physico-chemically and pharmacognostically.Flavonoids, alkaloids, glycosides, phenolic compounds and tannins, phytosterols and triterpenoids, quinones, and proteins were discovered in the ethanol extract after the plant was extracted using the maceration method. The leaves were subjected for pharmacognostical investigation which includes determination of physical constants such as ash value and extractive values. Macroscopic and microscopic characteristics of the leaves were studied. The extract's antifungal activity was assessed using the Sabouraud dextrose agar medium and the agar well diffusion method. Evaluation test for nail lacquer were performed and concluded that the nail lacquer passes the evaluation parameter.In ethanobotanical studies had proven that the *Tabernaemontana alternifoila* plant is used for treatment of

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onychomycosis. From this research we concluded that the plant have significant anti-fungal activity which will be useful for treatment of onychomycosis caused by fungus.

- *Conflict of Interest*

  Authors declare no conflict of interest.

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