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Experimental validation of folk medicinal knowledge used by 'Santal' tribes of Mayurbhanj District for the treatment of dermatophytosis

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Medicinal plants held a very specific space in every field related to the traditional health care system. Dermatophytosis, or ringworm, is a keratinophilic infection in humans, and approximately 25% of the world's population is affected by this disease. This is a contagious disease caused by a group of filamentous pathogenic fungi termed dermatophytes. Herbal drugs are used as the primary health care of the poor rural people, tribal and other indigenous communities because of the lack of modern health facilities. Though antibiotics and other synthetic drugs are more effective, they still rely on the healing properties of plant sources and the active compounds also proved to be beneficial for the dermatophytic species showing resistance to various synthetic antifungal drugs. An increase in fungal resistance leads to the need to find new potential alternatives which would be less toxic and more effective for the target disease. This study documents the indigenous use of traditional plants in the treatment of skin diseases by the indigenous community of three villages of the Mayurbhanj district of Odisha. Ten plant parts are collected from the survey areas and evaluated for their antifungal activity. Out of 10 folklore claimed plant sources, seed oils from three plant species, viz., *Millettia pinnata*, *Ricinus communis*, and *Talisia esculenta*, were validated experimentally for their antifungal activity against dermatophytosis caused by *Trichophyton mentagrophytes*. The objective behind this study is the scientific validation of the effectiveness of the indigenous knowledge, which may help in the discovery of new drugs.

Keywords: Antifungal activity, Dermatophytosis, Folklore claim, Santal and Khadia tribes, Seed oils

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Plants have always been invaluable companions to human society, fulfilling fundamental needs such as food, clothing, and shelter. The use of plants in medicinal practices to combat human pathogens has continued throughout eras. Natural ingredients play a significant role in healthcare systems, contributing to both internal and external treatments. Traditional Indian knowledge emphasizes that various parts of plants possess therapeutic properties for the treatment of diseases. The use of herbal medicine has been widely practiced for decades, particularly in rural communities where traditional knowledge is applied to address health issues[1]. This study presents information on wild traditional plants and their modes of application in the treatment of dermatophytosis.

Dermatophytosis is a superficial infection of the skin, commonly known as tinea, and the name varies depending upon the site of infection such as tinea corporis, capitis, tinea pedis, barbie, manum, cruris, and onychomycosis which is associated with the filamentous fungal species under the genera *Epidermophyton*, *Microsporum* and *Trichophyton*[2-5]. It is becoming prevalent among human beings and can damage the immune system of a completely healthy individual[6]. Dermatophytes can invade deeper tissues but generally prefer superficial keratinous tissues like non-living cutaneous and corneum layers for their growth and safety from the defence mechanisms of the host rather than due to an inability to invade deeper tissues[7].

Amphotericin B and echinocandins are the new methods included to treat dermatophytosis, whereas azole compounds such as itraconazole, ketoconazole, and terbinafine are mostly used to treat dermatophytosis[8]. The increased frequency of fungal infections is associated with the extensive use of antifungal drugs, resulting in resistance development. Finding natural new therapeutic alternatives to replace the synthetic compounds is mostly needed for the resistant microbial strains to avoid the harmful effects of the marketed antifungal formulations. Diversified culture and socio-religious practices, including the

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traditional medicinal system in the Indian subcontinent plays an important role in healthcare practices[9,10]. Complementary and Alternative Medicine (CAM) covers an exhaustive variety of treatments complemented with traditional practices along with the findings based on latest research to manage dermatophytosis[9].

India has four biodiversity hotspots and occupies in the 17 mega-biodiversity centres in the world[11]. According to the Botanical Survey of India (BSI), about 30,000 (two-thirds) plant species out of 45,000 species are important and valuable because of their medicinal values[12]. These plants are used as medicines for some diseases by the local people. The use of herbs to treat skin diseases is a well-established exercise in India[13]. In India, a number of plant species are used in managing the skin related ailments such as burns, acne, cuts, bruises, wounds, eczema, ringworm, scabies and leprosy. Local people not only use various plant parts like leaves, roots, fruit, and seeds for the care, protection and beautification of the skin but also use to treat different skin diseases[14]. Even *Cannabis indica* has showed anti-inflammatory, antipruritic, anti-ageing, and anticancer properties by various mechanisms, including interacting with the newly found endocannabinoid system of the skin and thereby providing a promising alternative to traditional treatments[15].

Onychomycosis is responsible for 50% of all nail infections[16,17]. It is the most frequent nail disease that occurs in adults and is caused mainly by fungal pathogens known as dermatophytes. These fungal pathogens have been categorised into seven main distinct genera, such as *Trichophyton*, *Epidermophyton*, *Microsporum*, *Nannizzia*, *Paraphyton*, *Arthroderma* and *Lophophyton*[18]. Onychomycosis has a global prevalence of 5.5% and a prevalence of 2-14% in the United States. The main risk factors include age, trauma, diabetes mellitus, peripheral artery disease, and genetic predisposition[19,20]. In Puerto Rico, studies are scarce, except for a panoramic view of dermatomycosis from 1930−1949, where 205 infections of onychomycosis were reported. The most predominant pathogens reported were *Trichophyton mentagrophytes* (68.8%), *T. rubrum* (30.20%), and only two cases of patients infected with *Epidermophyton floccosum*[18]. Literature data revealed that three fungal species such as *T. mentagrophytes*, *T. rubrum* and *E. floccosum* exhibited resistance to multiple classes of antifungal drugs, particularly showing complete resistance to terbinafine and significant resistance to various azoles, which establishes a multidrug resistance (MDR) phenotype and contributes to cross-resistance by decreasing intracellular drug accumulation, thereby reducing the efficacy of multiple antifungal agents[21].

The traditional health care system is an ultimate blessing for mankind from nature to treat their day-to-day problems and is the most trusted remedy to get in, whereas it introduces the plant-based medicinal knowledge effectively on healing many external infections, curing internal diseases, wounds, strengthening the immune system, and many more. The knowledge has been transferred over the years through repeated practices and communications. Hence, conservation and showcasing of this knowledge is a must to serve mankind with nature.

Materials and Methods

Survey area

An initiative was taken to conduct a survey work in three rural villages of Mayurbhanj district and had a one-on-one conversation with the villagers, who are really far from the influences of urbanization, to document the knowledge of traditional medicines. Prior informed consent was obtained from the ‘Sarapancha’ and all the informants of the village before conducting the survey. The survey was conducted in different seasons by making direct contact and conversation with the tribal communities. Three villages namely Balma, Manikapur, Upar-taladhia under Kundabai Panchayat of Mayurbhanj district of Odisha were selected to conduct the survey (Fig.1). Mayurbhanj

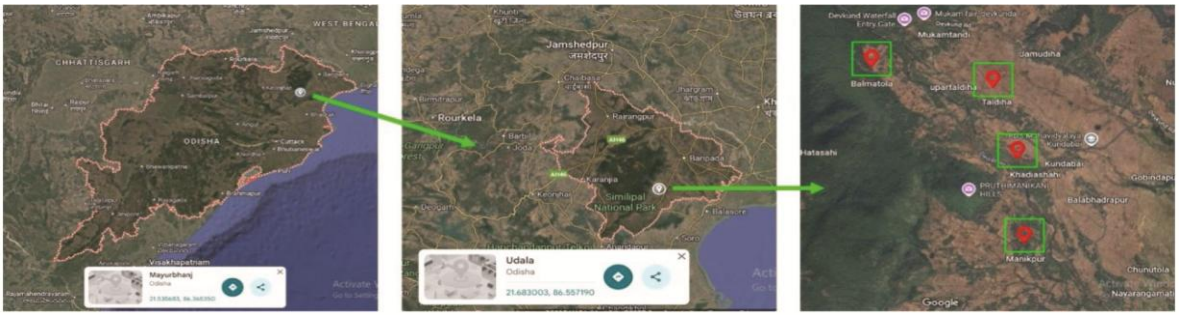


Fig. 1 — District Map showing the study area (Source: scribblemaps.com)

district is generally predominated by 'Santal tribes'. These three villages are inhabited by the Santal and Khadia tribes, but the documentation was carried out on the medicinal knowledge of Santal tribe as their population is dominated in Mayurbhanj districts than the Khadia tribe. Based on their opinion, "synthetic products can never work on what natural supplements can treat", they chose phytomedicine as a primary and preferable source rather than the synthetic formulations.

Collection and identification of plant sources

Information regarding the medicinal efficiency of different plant species to treat skin infections have been collected during the survey period by direct interaction with the tribal people (Table 1, Fig. 2 & Fig. 3). The use of plant parts and the mode of administration is also documented, which plays a vital role in the treatment process. The method of extraction, such as hot or cold, decoction or application of plant parts as a paste mask, is documented as well. Based on the information, the bark was collected from *Madhuca longifolia* var. *latifolia* (Roxb.) A.Chev and flowers were collected from both *M. longifolia* var. *latifolia* (Roxb.) A.Chev and *Thunbergia grandiflora* (Roxb. ex Rottler) Roxb. Fruits were collected from *Cassia fistula* L., *Diospyros melanoxylon* Roxb., *Millettia pinnata* L., *Talisia esculenta* (A.St.-Hil, A.Juss. & Cambess) Radlk., and *Ricinus communis* L., whereas pods and seeds were collected from *Butea monosperma* (Lam.) Taub. and *Jatropha gossypiifolia* L., respectively (Table 1). All the collected specimens were identified by Dr. Nabin Kumar Dhal, Chief Scientist, CSIR-IMMT, Bhubaneswar, and also cross verified with 'Flora of Orissa'.

Collection and culture of a fungal strain

The clinically infectious fungal strain, *i.e.*, *Trichophyton mentagrophytes* MTCC 7687, was collected from the microbiology department of KIMS Hospital, Bhubaneswar. As the basic fungal culture media, Sabouraud Dextrose Agar (SDA), did not support the growth of the *T. mentagrophytes*, therefore, on the basis of trial and error method, a specific medium was standardised by combining Potato Dextrose Agar (PDA) with SDA media in 2:1 ratio to culture these clinical isolates of *T. mentagrophytes* for conducting antifungal experiments. The culture was incubated at 38°C for 3 to 6 days for the desired growth.

Table 1 — Details of the eight folklore claimed plant sources

Common Name	Odia Name	Scientific Name	Family	Plant part used
Golden shower	Sunari	*Cassia fistula* L.	Fabaceae	Pods
Tendu	Kendu	*Diospyros melanoxylon* Roxb.	Ebenaceae	Seed
Bellyache bush	Jahazi gaba/ Kalajahaji	*Jatropha gossypiifolia* L.	Euphorbiaceae	Seed
Mahua	Mahula	*Madhuca longifolia* var. *latifolia* (Roxb.) A.Chev	Euphorbiaceae	Bark
Mahua	Mahula	*Madhuca longifolia* var. *latifolia* (Roxb.) A.Chev	Euphorbiaceae	Flower
Indian Beech Tree	Karanja	*Millettia pinnata* L.	Fabaceae	Seed
Castor	Jadaa	*Ricinus communis* L.	Euphorbiaceae	Seed
Pitomba	Kusuma/Pagada	*Talisia esculenta* Radlk.	Sapindaceae	Seed
Bengal clock vine	Neela lata	*Thunbergia grandiflora* (Roxb. ex Rottler) Roxb.	Acanthaceae	Flowers
Flame of the Forest	Palasah	*Butea monosperma* (Lam.) Taub.	Fabaceae	Pods

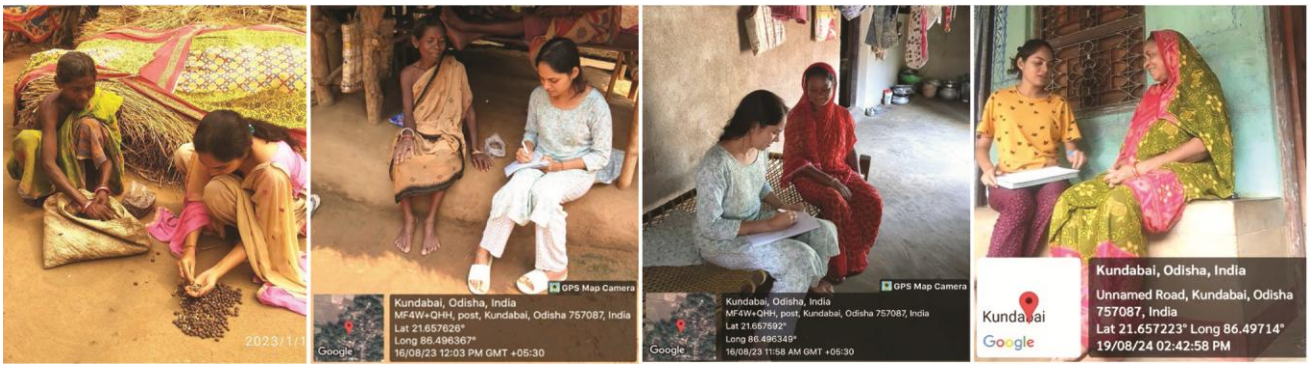


Fig. 2 — Interaction and collection of different plant sources from tribal communities

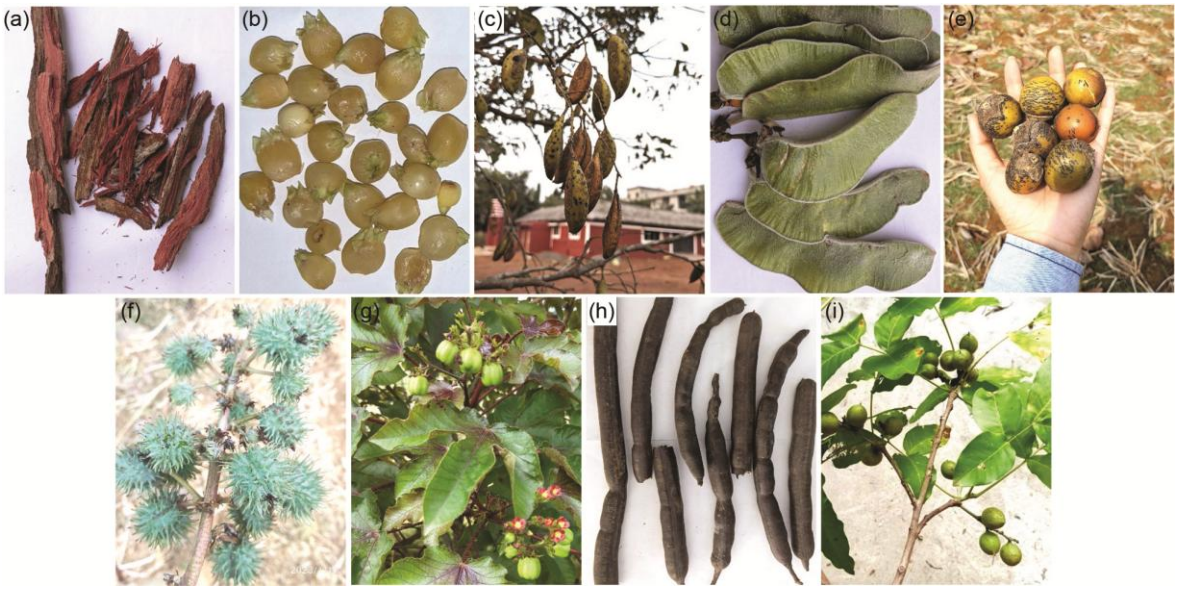


Fig. 3 — Collected raw plant samples from villages during survey work displays (a)bark & (b) flowers of *M. indica*, Pods of (c) *M. pinnata*, (d) *B. monosperma*, Fruits of (e) *D. melanoxylon*, (f) *R. communis*, (g) *J. gossypiifolia*, (h) *C. fistula* and (i) *T. esculenta*

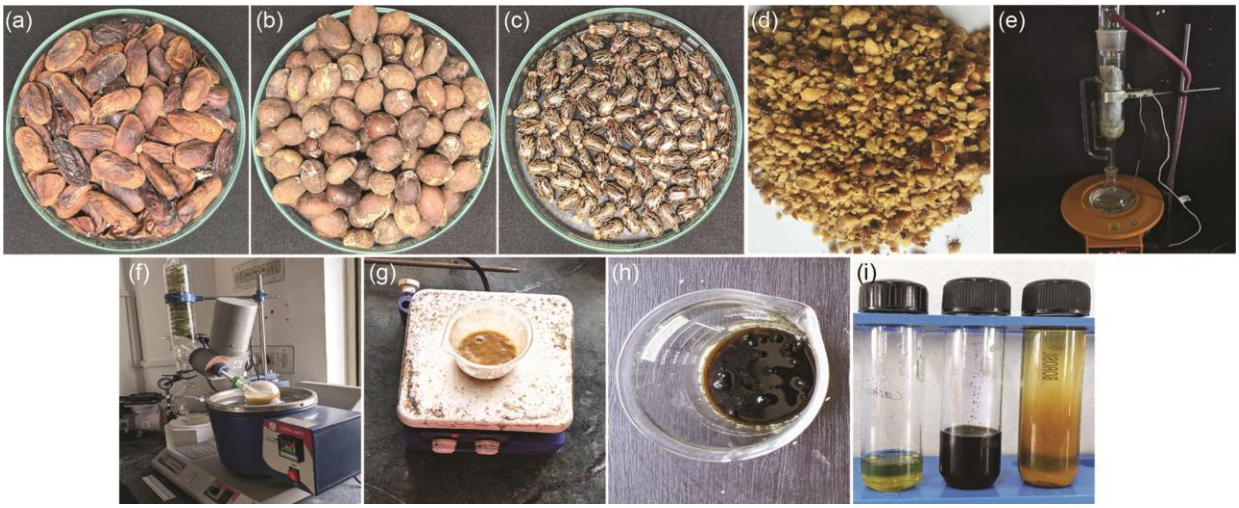


Fig. 4 — (a) *M. pinnata*, (b) *T. esculenta*, (c) *R. communis* dried seed samples taken for oil extraction; Oil extraction in laboratory conditions using (d) crushed seed, (e) Soxhlet apparatus for water extraction, (f) Evaporation using a rotary evaporator, (g) heating of the evaporated extract using a heating metal plate, (h) unfiltered oil sample, and (i) Extracted oils of three different seed samples

Preparation of oil extractions

The collected seed samples from *R. communis*, *T. esculenta*, and *M. pinnata* (Fig. 4 (a-c) were subjected to extraction and evaporation using a Soxhlet apparatus and a rotary evaporator[22], respectively. The extracted oil (Fig. 4 (d-i)) from the three seed samples was taken to study their antimicrobial activity against the onychomycotic fungus *T. mentagrophytes*.

XRF analysis

Two millilitres of each sample from all the selected plants were analysed for their mineral content by using the X-ray fluorescence spectroscopy method at the Advanced Testing Laboratory of Centurion University of Technology and Management, Bhubaneswar campus, to find out the different minerals that are useful to improve human skin health.

Results

Documentation of tribal knowledge

The anti-dermatophytic activity of the plant sources, preparation methods, and mode of administration used by the inhabitants of Balma, Kundabai, and Upartaldhia villages of Mayurbhanj district of Odisha are mentioned in (Table 2).

Table 2 — Method of preparation and mode of administration of plant resources against skin diseases

Scientific Name	Plant part used	Method of Preparation	Mode of administration	Skin ailments
C. fistula L.	Dry pods	Dry pods are ground using either a stone pestle (local name '*Sila*' or a traditional husking lever (local name '*Dhinki*'), resulting in a black gummy paste, which is soaked in water for about 4 to 5 days in an earthen pot. Then it is allowed for extraction using a clean cloth, and the residue is discarded. The extracted solution was kept on fire to remove excess water content.	The crude extract is applied on infected skin	used to get relief from skin infections and eczema
D. melanoxylon *J. gossip iifolia* *M. pinnata* *R. communis* *T. esculenta*	Seed	Seeds were made into coarse powder, then water was added and allowed to boil in a low flame overnight, and oil was extracted after cooling using a clean cotton cloth	seed oil is applied on infected skin, nail and other epidermal parts	used to get relief from skin and nail infections
	Bark	Collected dried bark samples are allowed to boil with water for at least 7 to 8 hours over a fire.	Extracted bark water is used in bathing	used to get relief from chicken pox, skin rashes, and relief from body pain
M. longifolia	Flower	Juice is extracted by two methods, either from the fermentation process of dry flowers or from fresh extraction of the flowers	Fermented raw flower juice is applied externally on whole body	used for skin rejuvenation, detoxification, and to avoid any skin infection
B. monosperma	Fresh green pods	Fresh green pods are ground using either a stone pestle (local name '*Sila*' or a traditional husking lever (local name '*Dhinki*')	Paste is applied on the scalp and kept for 2-3 hours, and then washed off	used to get relief from dandruff and other scalp infections

Mineral content by X-ray fluorescence spectroscopy (XRF) analysis

The XRF analysis from the three oil samples revealed the presence of 14 mineral compounds (Table 3). Whereas; phosphorus (P), sulphur (S), chlorine (Cl), potassium (K), calcium (Ca), iron (Fe), tin (Sn), and erbium (Er) were found in *T. esculenta*; phosphorus (P), chlorine (Cl), calcium (Ca), iron (Fe), copper (Cu), zirconium (Zr), and terbium (Tb) were found in *R. communis* and phosphorus (P), sulphur (S), chlorine (Cl), calcium (Ca), iron (Fe), tin (Sn), zinc (Zn), zirconium (Zr) europium (Eu) and rhenium (Re) were detected from *M. pinnata* (Table 3). Out of all, P is detected with the highest amount from *T. esculenta* and *R. communis*; however, S is highest from *M. pinnata* (Fig. 5).

Table 3 — Name of mineral compounds present in the three oil contents

Mineral List	*T. esculenta*	*M. pinnata*	*R. communis*
P	629.9	334.5	349.4
S	310	879.3	-
Cl	135.3	79.4	50.2
Ca	120.2	138.5	117.8
Fe	20.1	24.1	17.8
Zn	-	2.5	-
Zr	-	6.2	6.8
Sn	43.1	38	-
Eu	-	19	-
Re	-	0.3	-
Er	89.4	-	-
Cu	-	-	2.5
Tb	-	-	69.4
K	262.8	-	-

Antifungal activity

To evaluate the effectiveness of ancient medicinal knowledge, the extracted samples were tested for their antimicrobial efficacy against pathogenic clinical isolates, *T. mentagrophytes*, cultured from a sample collected from a patient suffering from dermatophytosis at KIMS Hospital, Bhubaneswar.

The *T. mentagrophytes* strain was cultured in laboratory conditions and tested against a marketed antifungal medicine as a positive control. Whereas the three different oil samples are also observed against *T. mentagrophyte* under aseptic conditions to obtain the result. The fungal pathogen *T. mentagrophytes*

was cultured in standardized media combined with PDA and SDA in the ratio 2:1 at 38°C for 3 to 6 days. All the selected plant samples mentioned in (Table 1) were prepared and experimented against the causal organism *T. mentagrophytes*; whereas, the three oil samples showed effective inhibition (Table 4). The antifungal plates were observed at an interval of every 3 days to ensure the quality of the culture (Fig. 6), because the fungal specimen takes a longer period to grow. After all, *M. pinnata*, *T. esculenta*, and *R. communis* resulted positively, which indicates the impactful effect of natural plant supplements in the treatment of dermatophytosis. Two marketed antifungal medicines called terbinafine and ketoconazole were also compared which resulted a negative effect (Fig. 6). Positive control such as terbinafine and ketoconazole are the standardised or prescribed drugs which should inhibit the pathogen, but in this study, these two prescribed drugs showed negative result, which indicates that the particular pathogenic strain might be multi drug resistant (MDR).

Discussion

Research based on plant-based supplements works as a blessing for the rural villagers to overcome their dermatophytic medicinal needs. They use different plant parts such as leaf, root, bark paste, and bark boiled with water, seed oil, to get rid of the dermatophytic infections. The juice of the mahua flower is claimed to be effective on skin health and is massaged for oleation[23]. *C. fistula* fresh leaf extract is effective for the management of fungal skin infections caused by *C. albicans* in some laboratory-cultured specimens[24].

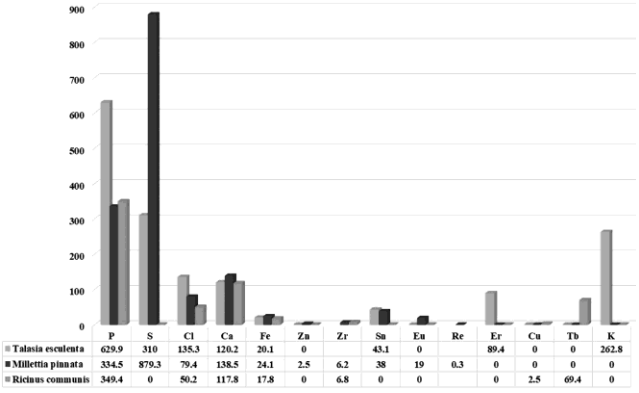
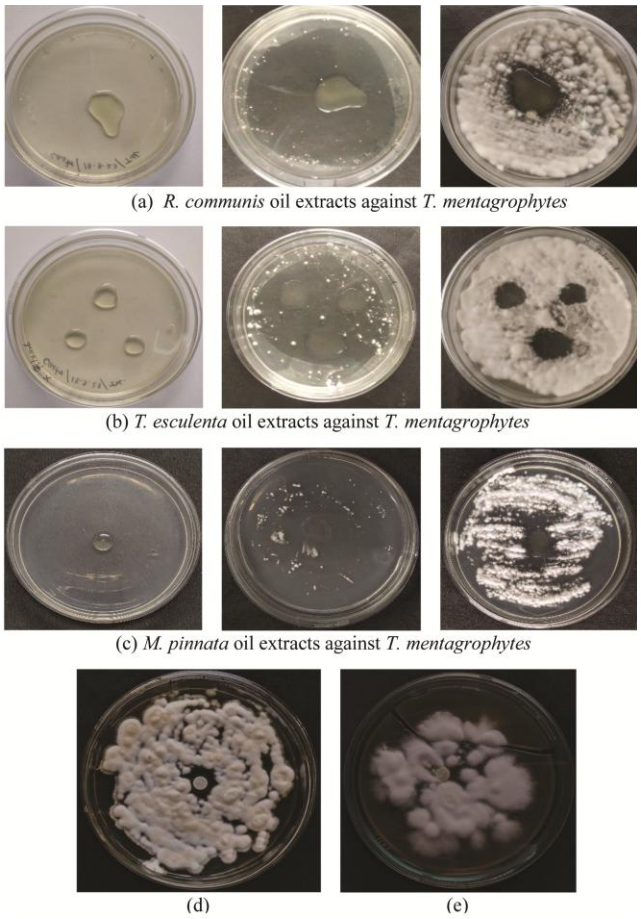


Fig. 5 — Mineral contents in three seed oils



(a) *R. communis* oil extracts against *T. mentagrophytes*

(b) *T. esculenta* oil extracts against *T. mentagrophytes*

(c) *M. pinnata* oil extracts against *T. mentagrophytes*

(d) (e)

Fig. 6 — Antifungal activity of extracted oil samples against *T. mentagrophytes* (a,b,c) and marketed antifungal drug supplements, (d) Terbinafine and (e) Ketoconazole

Table 4 — Different plant extractions showing results against *T. mentagrophytes*

Scientific Name	Plant part used	Extractive solvent	Antifungal activity
C. fistula	Pod	Water, ethanol	-Ve
D. melanoxylon	Seed	Petroleum ether	- Ve
J. gossypiifolia	seed	Ethanol, water	-Ve
M. longifolia	Bark	Water, ethanol	- Ve
M. longifolia	Flower	Water, ethanol	- Ve
M. pinnata	Seed	Petroleum ether	+Ve
R. communis	Seed	Petroleum ether	+Ve
T. grandiflora	Flowers	Water, ethanol	- Ve
T. esculenta	seed	Petroleum ether	+Ve
B. monosperma	Pod	Water, ethanol	-Ve

Distinct elemental specificity was also observed. Potassium (K) was exclusively detected in *T. esculenta*, indicating possible selective uptake or soil-specific enrichment. Similarly, zinc (Zn) was found only in *M. pinnata*, while copper (Cu) was unique to *R. communis*. The presence of zirconium (Zr) in both *R. communis* and *M. pinnata* suggests partial overlap in geochemical characteristics, whereas rare earth elements (REEs) such as erbium (Er), terbium (Tb), europium (Eu), and rhenium (Re) were distributed differentially across the three oils, highlighting their potential as geochemical markers. Mineral nutrients such as phosphorus (P) and sulphur (S) play critical roles in plant defence mechanisms, which influence a plant's ability to resist fungal pathogens. and can indirectly contribute to antifungal activity through multiple biochemical and physiological pathways. When compared with previous studies on edible oils such as olive oil, the concentration of trace elements in the present samples appears consistent with the general observation that trace elements typically occur at very low levels, often in the range of μg/kg or ng/g. REEs in olive oil occur in ultra-trace quantities, reinforcing the sensitivity of XRF in detecting such elements[25,26]. The variability in elemental composition can be attributed to multiple factors, including cultivar type, fertilizer application, soil composition, and climatic conditions[27-31].

Methanolic dried leaf extract of *R. communis* shows antifungal effect against *Curvularia*, *Microsporum*, and *Penicillium*[32], which co-relates to this finding that the seed oil also has antifungal activity against the selected fungus *T. mentagrophytes*, implying that various sources of this plant have antifungal activity against diverse fungal species. Antifungal activity of *D. melanoxylon* bark extract (ethanol, Methanol, water, petroleum ether, and ethyl acetate) collected from the Similipal biosphere reserve, showing good results in inhibiting four *Candida* species[33]. Not only in India, but also some essential medicinal plants, such as *Tinospora crispa*, *Andrographis paniculata*, and *Centella asiatica*, are used to treat skin infections in Indonesia. It claims that using traditional medicine in this modern health care concern may introduce a sustainable and alternative way for treatment for future generations[34].

However, to treat skin treatments the seed oil works effectively amongst all because it remains for a longer time on the applied area and thereby helps in hydrating the infected area, balances the moisture content of the skin, inhibiting the dryness, thereby enhancing the immunity of the skin against fungal infection. The villagers prefer oil, or oil-based formulations, to apply externally on the infected body parts because of its sticky nature, as most of the time they are engaged in diversified working fields such as agriculture, forest dwelling, and fishing for their livelihood. Besides hydration, spread ability, and retention for a longer period, oil can be applied as an effective product that contains natural healing properties because of the presence of secondary metabolites having antioxidant characteristics. The present study focuses on the traditional use of seed oil and oil-based formulations in the treatment of dermatophytosis among tribal inhabitants of the study area. This study has few limitations. The efficacy of the seed oil was evaluated based on ethno-medicinal knowledge and local practices without clinical validation. Further studies to be focused on isolation and characterization of the active metabolites responsible for the treatment of dermatophytosis

Conclusion

The present work shows an advancement in using traditional knowledge in its raw form to treat dermatophytosis induced by *T. mentagrophytes*. Out of 10 selected plant extracts, the extracted oil showed positive results in controlling the growth of potential MDR fungal pathogen. From this work, it is confirmed that the villagers are still depending on the plants and plant-based products to fulfil their medicinal needs against different diseases, including dermatophytosis. This work is focused on the experimental validation of the folklore claims of the native plant sources, especially the seed oil of *R. communis*, *M. pinnata*, and *T. esculenta* used in curing onychomycosis caused by *T. mentagrophytes*.

Acknowledgments

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Author Contributions

Both authors contributed to conception and design. Material preparation, data collection, sample collection, and the laboratory experiments and analysis were performed by M G. The manuscript draft and editing were prepared by S P. Both authors read and approved the final manuscript.

Conflict of Interest

None of the authors has a conflict of interest to disclose.

Prior Informed Consent

Prior informed consent was obtained from the Sarapancha of the villages.

Ethics Approval

The present study involved plant materials only and did not involve human participants or animal experimentation. Therefore, ethical committee approval was not required.

Biosafety

The experiments involving the *T. mentagrophytes* strains were carried out adhering to the standard Biosafety in Microbiological and Biomedical Laboratories (BMBL) guidelines.

Data Availability

Ethnomedicinal data will be automatically made available to the public domain once published.

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