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Antifungal Activity of Crude *Solanum incanum* Fruit Extracts Against *Trichophyton mentagrophytes* and Exploratory Analysis of Virulence-Associated Gene Expression

Pita Deta[1]*, Peter Ogoti[2], Daniel Kiboi[2], and Christine Bii[3]

[1]*Pan African University Institute for Basic Sciences, Technology and Innovation (PAUSTI), Nairobi, Kenya*
[2]*Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya*
[3]*Kenya Medical Research Institute (KEMRI), Nairobi, Kenya*

*Corresponding author's Email: pitadeta5@gmail.com

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ABSTRACT

Dermatophyte infections in humans and animals are a significant public health concern, and increasing resistance to antifungal medicines highlights the need to explore alternative therapeutic agents. The present study aimed to preliminarily assess the *in vitro* antifungal activity of crude extracts from *Solanum incanum* (*S. incanum*) fruit against *Trichophyton mentagrophytes (T. mentagrophytes)*. *Solanum incanum* fruits were extracted with hexane, ethyl acetate, methanol, and water. The levels of phenolic, flavonoid, and alkaloid compounds were measured, and antifungal activity was assessed using disc diffusion, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) assays. Methanol and aqueous extracts exhibited the highest phenolic contents (~19 mg Gallic Acid Equivalents/g extract) and demonstrated significant antifungal activity, producing inhibition zones of 14.46 mm and 8.57 mm, respectively, at a concentration of 5 mg/mL. However, terbinafine demonstrated marginally greater inhibitory activity (15.02 mm) at a lower concentration of 0.1 mg/mL. The present findings indicated that using methanol and aqueous extracts of *S. incanum* fruit was associated with lower transcript levels of the targeted virulence-related genes (*SUB3* and *StuA*). The current findings provided preliminary evidence regarding the antifungal activity of *S. incanum* fruit extract against *T. mentagrophytes* at a concentration of 5 mg/mL, indicating its potential as an antifungal agent.

Keywords: Antifungal activity, Bioactive compound, Dermatophyte, Gene expression, Medicinal plant

INTRODUCTION

Over the past twenty years, there has been a notable increase in both superficial and systemic fungal infections, many of which pose significant public health concerns (Richardson, 2022). The increase is particularly noted among individuals with compromised immune systems, including those living with Human Immunodeficiency Virus (HIV), patients undergoing cancer chemotherapy, and individuals receiving extended courses of antibiotic treatment (Bongomin et al., 2017). Dermatophytes, a type of filamentous fungus, are the main cause of superficial mycoses and colonize keratinized tissues such as the skin, nails, and hair (Weitzman and Summerbell, 1995). Dermatophyte infections are generally limited to the skin; however, dissemination to other keratinized or extra-cutaneous tissues may occur in immunocompromised individuals, resulting in more severe clinical manifestations and substantial health complications (Bongomin et al., 2017).

Trichophyton mentagrophytes (*T. mentagrophytes*) is a zoophilic dermatophyte and a major causative agent of dermatophytosis (ringworm) in a wide range of animal species (Paryuni et al., 2020). *Trichophyton mentagrophytes* frequently infects companion animals such as dogs and cats, as well as livestock, including cattle, sheep, and goats, and several rodent species (Paryuni et al., 2020). In veterinary medicine, dermatophytosis represented a significant health and economic burden (Kappes et al., 2023). In livestock, infections can result in reduced weight gain, decreased productivity, and damage to hides and wool, thereby lowering their market value (Kappes et al., 2023). Furthermore, the disease increases expenses for treatment, control efforts, and farm management (Kappes et al., 2023). Notably, *T. mentagrophytes* is zoonotic and can be transmitted from animals to humans, posing occupational risks to farmers, veterinarians, and animal handlers (Plangsiri et al., 2025). The management of dermatophytosis in animals remains challenging due to the limited availability of licensed veterinary antifungal medications, the emergence of antifungal resistance, and concerns associated with drug residues and withdrawal periods in food-producing animals (Frymus et al., 2013).

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Fungal skin infections affect approximately 25% of the world's population, making them some of the most common infectious diseases in humans. *Trichophyton* species are the leading cause of dermatophytosis, accounting for about 70% of cases (Bongomin et al., 2017). Although antifungal agents such as azoles and allylamines are available, their use is often limited by resistance, toxicity, high costs, and limited access in low-resource settings (Perlin et al., 2017). These limitations highlight the urgent need for safer, more affordable, and more effective antifungal agents. Traditional medicine plays a vital role in healthcare systems across Africa, with around 80% of the population relying on herbal medicines for their well-being (WHO AFRO, 2003). *Solanum incanum* (*S. incanum*), commonly known as Sodom's apple, plays a crucial role in African ethnomedicine for treating several diseases, including skin infections such as ringworm, dermatitis, and wounds (Kokwaro, 2009; De Wet et al., 2013). Several studies have demonstrated that extracts of *S. incanum* exhibited antibacterial, analgesic, and anti-inflammatory activities, as reported in experimental studies evaluating its pharmacological properties (Sbhatu and Abraha, 2020; Musyimi et al., 2021). However, despite comprehensive ethnobotanical documentation, the antifungal efficacy of *S. incanum* against dermatophytes, particularly those caused by *T. mentagrophytes*, remains inadequately investigated.

Previous qualitative phytochemical studies of *S. incanum* have consistently identified several major classes of secondary metabolites, such as flavonoids, alkaloids, tannins, and saponins (Mshimba, 2014). The complex phytochemical profile of *S. incanum* suggested potential pharmacological properties that may explain its traditional therapeutic uses.

Understanding the mechanism of antifungal action is essential for scientific and clinical advancement. The pathogenicity of *Trichophyton* spp. is linked to increased expression of several virulence genes, including secreted proteases such as *subtilisin 3* (*SUB3*), which facilitate host tissue degradation and invasion (Martínez-Rossi et al., 2018). In addition, transcriptional regulators such as *StuA* play a key role in stress tolerance, fungal growth, and virulence regulation (Gauthier et al., 2010; Lang et al., 2020). Previous studies have demonstrated that natural compounds can modulate fungal gene expression (Silva et al., 2016). For instance, eugenol, an antifungal compound derived from plants, notably downregulated the SUB3 gene in *T. rubrum*, with effects comparable to those observed with terbinafine treatment (Silva et al., 2016). The assessment of gene expression profiles provides a molecular approach to evaluating the ability of plant extracts to disrupt fungal virulence pathways and elucidating the mechanisms of antifungal action (Ball et al., 2020). The present study aimed to assess the antifungal properties of *S. incanum* extracts against *T. mentagrophytes* and to investigate their potential effects on virulence-related genes.

MATERIALS AND METHODS

Ethical approval

The study was conducted in accordance with the ethical principles and approved procedures of the Institutional Scientific and Ethical Review Committee at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya. Ethical approval for the study was obtained from the committee before the study began under the approval number JKU/ISERC/02316/1907. All experimental procedures were performed in compliance with the approved institutional guidelines and ethical standards.

Solanum incanum collection

A total of 4 kg of whole *S. incanum* plants were collected from the Mungetho agricultural area of Juja Farm in Kiambu County, Kenya, for identification, and approximately 2 kg of yellow fruits were used for extraction. The verification of *S. incanum* plants was conducted at the Botany Laboratory of JKUAT, Kenya. A deposit of *S. incanum* plants has been made in the herbarium under accession number PSD-JKUATBH/001.2/2025.

Plant extracts preparation

The fruits of *S. incanum* were washed with distilled water and air-dried at room temperature (25°C) for nine days. The yellow outer pericarp was removed, and the seeds were retained for extraction. The dried fruits of *S. incanum* were ground into powder using an electric grinder. About 100 g of this powdered material was extracted sequentially with solvents of increasing polarity, hexane, ethyl acetate, and methanol, to obtain different fractions from the same plant source. In addition, an aqueous extract was prepared independently to mimic traditional usage and to evaluate water-soluble bioactive compounds not fully represented in organic solvent fractions. The powdered substance was immersed in 1000 mL of hexane inside a conical flask sealed with a rubber stopper and left for 72 hours away from direct sunlight, with occasional shaking. Then, sterile filter paper was used to filter the mixture, and a rotary vacuum evaporator was used to concentrate the filtrate at 42°C. The residue was re-immersed in 1000 mL of ethyl acetate and left in the dark for five days, then filtered and concentrated at 42°C. The remaining residue was re-soaked in 1000 mL of methanol for 72

hours under the same conditions, then filtered and concentrated at 65°C under reduced pressure to prevent damage to thermolabile compounds. For the aqueous extraction procedure, 100 g of the powder was suspended in 1000 mL of distilled water, heated at 50°C for a duration of 20 minutes, then cooled, and subsequently kept at 25°C with agitation for a period of 24 hours. Following filtration, the extract was stored at −80°C for 72 hours prior to lyophilization (freeze-drying; Raghuvanshi and Gupta, 2023). The dried *S. incanum* extracts were weighed, recorded, and stored at 4°C until further analysis. The extraction yield (%) was calculated using Formula 1.

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100 \quad \text{(Formula 1)}$$

Dermatophytes strain isolation

Trichophyton mentagrophytes strain was obtained from the Kenya Medical Research Institute (KEMRI) Mycology Laboratory's culture collection in Nairobi, Kenya, and was initially isolated from an infected patient in 2013. Before experimentation, the isolate was subcultured under standard laboratory conditions, and its identity was reconfirmed by microscopic examination with lactophenol cotton blue staining. Morphological features were examined with an light microscope (Olympus, Japan) to verify characteristic fungal structures before use in experiments. The isolate was subsequently subcultured on Sabouraud dextrose agar (SDA) following standard mycological procedures (De Hoog et al., 2020). Inoculated plates were incubated at 30°C for six days under controlled laboratory conditions, with regular monitoring of fungal growth.

Quantitative screening of flavonoid, alkaloid, and phenolic content

Total flavonoid content

A quercetin standard stock solution (1 mg/mL) was prepared by dissolving 10 mg of quercetin in 10 mL of methanol. This stock solution was then diluted with distilled water to produce working standard solutions at concentrations of 0, 10, 20, 30, and 40 µg/mL. A calibration curve was established by plotting absorbance at 510 nm against quercetin concentration. Total flavonoid levels in the *S. incanum* extracts, including hexane, ethyl acetate, methanol, and aqueous fruit extracts, were measured using the aluminum chloride colorimetric method (Chang et al., 2002). For each extract (1 mg/mL), 1 mL was mixed with 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$. After five minutes, 0.3 mL of 10% $AlCl_3$ was added, and the mixture sat for an additional six minutes. Then, 2 mL of 1 M NaOH was added, and the total volume was adjusted to 10 mL with distilled water. Absorbance was measured at 510 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan). A reagent blank containing all assay reagents except the extract, which was replaced with the corresponding solvent, was employed to calibrate the spectrophotometer and rectify background absorbance. The total flavonoid content was determined from the quercetin calibration curve as depicted in Formula 2.

$$\text{TFC} = \frac{C \times V}{m} \quad \text{(Formula 2)}$$

Where C indicated the concentration of flavonoids from the calibration curve (as quercetin equivalent), V was the volume of extract used in the assay (mL), and m represented the mass of extract used (g), expressed as mg Quercetin Equivalents (QE)/g extract.

Total alkaloid content

The acid-base precipitation method was used to estimate the total alkaloid concentrations in *S. incanum* fruit extracts. Briefly, 0.1 g of the extract was placed in a 250 mL beaker, and 200 mL of 10% acetic acid in ethanol was added. The mixture was covered and allowed to stand at room temperature (25°C) for four hours (Harborne, 1998). The solution was then filtered, and the filtrate was concentrated in a water bath maintained at 50°C until the volume was reduced to one-quarter of its original volume. Concentrated ammonium hydroxide was then added dropwise to the concentrated extract until complete precipitation was achieved. The mixture was left undisturbed to allow the precipitate to settle completely. The precipitated alkaloids were then collected by filtration, rinsed with dilute ammonium hydroxide, dried to a constant weight, and weighed. A reagent blank was prepared following the same procedure, but without the plant extract. The alkaloid mass was calculated according to Formula 3.

$$m_{alkaloid} = m_{total} - m_{container} - m_{blank} \quad \text{(Formula 3)}$$

Where m total represented the mass of the container with all residues (g), m container was the mass of the empty container (g) used to hold the extract, and m blank represented the mass of residues arising from solvent impurities (g).

Total alkaloid content (TAC) was calculated using Formula 4, based on an initial extract mass of 0.1 g.

$$\text{TAC (\%)} = \frac{\text{Mass of alkaloid}}{\text{Initial extract mass}} \times 100 \quad \text{(Formula 4)}$$

Alkaloid content was expressed as a percentage (%) of the dry extract based on the gravimetric determination method, whereas total flavonoid content was expressed in mg/g using a standard calibration curve.

Total phenolic content

Total phenolic concentrations of the four different extracts were measured using the Folin-Ciocalteu colorimetric method (Ainsworth and Gillespie, 2007). A gallic acid stock solution (1 mg/mL) was prepared by dissolving 10 mg of gallic acid in 10 mL of distilled water. Working standard solutions (0, 10, 20, 30, 40, 50 µg/mL) were created through serial dilution of this stock. During the assay, 0.5 mL of each extract (1 mg/mL) or standard solution was added to a test tube, then mixed with 2.5 mL of diluted Folin-Ciocalteu reagent. The mixture was vortexed thoroughly and incubated at 25°C for 10 minutes. Then, 2 mL of 7.5% sodium carbonate (Na_2CO_3) solution (Sigma-Aldrich, St. Louis, MO, USA) was added, vortexed, and incubated in the dark at room temperature (25°C) for 60 minutes. Absorbance was measured at 760 nm using a UV–Visible spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan) against a reagent blank containing all assay reagents except the plant extract, which was replaced with the corresponding solvent. The working reagent was prepared by diluting the Folin-Ciocalteu reagent 1:1 with distilled water, and a 7.5% (w/v) sodium carbonate solution was prepared by dissolving 7.5 g of Na_2CO_3 in 100 mL of water, yielding a total reaction volume of 10 mL, corresponding to 0.5 mg of extract per assay. Total phenolic content (TPC) was determined using the gallic acid calibration curve, according to Formula 5.

$$TPC = \frac{C \times V}{m} \quad \text{(Formula 5)}$$

Where C was the concentration from the calibration curve (as gallic acid equivalent), V represented the volume of extract used (mL), and m indicated the mass of extract used (g), and was expressed as mg gallic acid equivalent per gram of extract (mg GAE/g extract).

***In vitro* antifungal activity of *Solanum incanum* fruit extracts**

A stock solution of 1000 mg/mL was prepared in 100% Dimethyl sulfoxide (DMSO) with vigorous vortexing to ensure maximum dissolution. Working solutions (5 mg/mL) were prepared by diluting the stock with Sabouraud Dextrose Broth (SDB), ensuring that the final DMSO concentration was below 1% (v/v). No visible precipitation was observed at the working concentrations used in the assays. Conidia were collected using sterile saline containing 0.05% Tween 80, filtered to remove hyphal fragments, and adjusted to approximately 1×10^6 conidia/mL with a hemocytometer, following CLSI (2008) guidelines. Sterile SDA plates were inoculated by evenly swabbing the standardized suspension to create a uniform lawn. Sterile Whatman No. 1 filter paper discs (6 mm diameter) were soaked with 10 µL of plant fruit extracts and then dried aseptically (CLSI, 2008). The discs were placed on the inoculated agar with sterile forceps. Discs containing terbinafine served as positive controls, while discs containing 1% DMSO and distilled water served as negative controls. Plates were incubated at 30°C for 5 days, and the inhibition zone diameters were measured in millimeters (mm) with a digital caliper.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the methanolic and aqueous fruit extracts was ascertained utilizing the broth microdilution technique (CLSI, 2008). Two-fold serial dilutions of each extract were prepared in sterile SDB, starting at 10 mg/mL and extending through 10 successive dilutions. Each well of a sterile 96-well microtiter plate contained a final volume of 200 µL, consisting of the plant extract, SDB, and fungal inoculum. Control wells included SDB with fungal inoculum only for growth assessment, while sterile control wells contained SDB without inoculum. Terbinafine was incorporated as a positive control under identical conditions. The fungal inoculum was standardized to a final concentration of 1×10^5 conidia/mL in each well. The plates were incubated at 30°C for five days and monitored daily for fungal growth. The MIC was defined as the lowest concentration of the extract required to prevent visible fungal growth after five days, relative to the control. The assay was carried out in three independent experiments, each performed in triplicate, and the results were expressed in mg/mL.

Minimum fungicidal concentration

To determine the minimum fungicidal concentration (MFC), 10 µL aliquots were aseptically taken from wells that exhibited no visible growth in the MIC assay at different concentrations, from growth control wells on control plates, and from sterile control wells, and then subcultured onto SDA plates. The plates were then incubated at 30°C for five days and monitored daily for fungal growth. The MFC was defined as the lowest extract concentration that exhibited no visible colony growth on agar plates after five days of incubation (Scorzoni et al., 2007). The assay was conducted in three independent experiments, each performed in triplicate, and the results were reported in mg/mL.

Culture conditions and exposure to *Solanum incanum* extracts

Trichophyton mentagrophytes inoculum was prepared as a conidial suspension adjusted to 1×10^6 conidia/mL using a hemocytometer (Neubauer improved chamber, Marienfeld-Superior, Germany). The 5 mL fungal suspension was

incubated at 30°C with gentle shaking (120 rpm) for seven hours to facilitate conidial germination and the development of metabolically active hyphae before treatment. Cultures were then treated with *S. incanum* extracts at their respective MICs (Cowen et al., 2015) and incubated at 30°C for an additional 12 hours, providing a biologically relevant period to evaluate the expression of genes related to growth and virulence (Balouiri et al., 2016). Untreated cultures maintained under identical conditions served as negative controls.

RNA extraction and quality assessment

Total RNA was extracted from 60 mg of fungal mycelia using the Illustra RNAspin Mini RNA Isolation Kit (GE Healthcare, Little Chalfont, United Kingdom), according to the manufacturer's instructions. Each experimental condition consisted of three independent biological replicates. An on-column DNase I digestion step was included to eliminate genomic DNA contamination. The RNA concentration and purity were assessed using UV spectrophotometry (NanoDrop, Thermo Fisher Scientific, USA), with acceptable A260/A280 ratios ranging between 1.8 and 2.1. The RNA integrity was confirmed by electrophoresis on a 1.2% denaturing agarose gel, which revealed distinct 28S and 18S rRNA bands with minimal degradation. Only pure, high-quality RNA samples were used for subsequent analysis.

Quantitative real-time PCR

Gene expression analysis was conducted on two virulence-associated genes (*SUB3* and *StuA*) as well as two reference genes (*β-tubulin* and *GAPDH*). The complementary DNA (cDNA) was synthesized from 1 µg of total RNA within a 20 µL reaction volume, utilizing the RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Vilnius, Lithuania) in accordance with the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was carried out in a total volume of 20 µL, which included 10 µL of SYBR Taq ReadyMix (Sigma-Aldrich, USA), 0.5 µM of each primer, 4 µL of cDNA template, and 5 µL of nuclease-free water. Amplifications were conducted on an Mx3300P QPCR System (Agilent Technologies, Stratagene, USA) under the following conditions. The initial denaturation at 95°C for one minute was followed by 40 cycles at 95°C for 15 seconds and at 60°C for 60 seconds. A melt curve analysis (60-95°C) was performed at the end of each run to confirm amplification specificity (Bustin et al., 2009). No-template controls (NTC) and no-reverse transcription controls (no-RT) were included to verify the absence of contamination and genomic DNA. Untreated *T. mentagrophytes* cultures were used as the calibrator condition. Each sample was analyzed with three biological replicates, each with three technical replicates. Primer efficiency (90-110%) was validated using standard curves before analysis (Liu et al., 2023). Delta cycle threshold (ΔCt) values were calculated for each biological replicate by subtracting the geometric mean cycle threshold (Ct) of the reference genes (*GAPDH* and *β-tubulin*) from the Ct of the target gene. Delta Delta Ct (ΔΔCt) values were then calculated relative to the untreated control group. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$, with normalization performed using the geometric mean of *β-tubulin* and *GAPDH* Ct values, following validation of their expression stability (Livak and Schmittgen, 2001).

Table 1. Primers used for qRT-PCR in the present study

Gene	Sequence	Size (bp)	Reference number
SUB3	F: 5′- TCATGAAGGACGGTGTCACT -3′ R: 5′- ACGCTTAGCCTTGTTGAGGT -3′	104	KF_146901.1
StuA	F: 5'- CGTCCTGAAACCAGCGAAGA -3' R: 5'- ACCCATGTCACGATGCCATA -3'	98	XM_003010308.1
GAPDH	F: 5'- TCATCTCTGCTCCCTCTGCT -3' R: 5'-GACGGTGGTCATCAAACCCT-3'	174	MW_959142.1
β-tubulin	F: 5'- CGACCAAGACCCTTCCCTTC -3' R: 5'-GACTGACCGAAGACGACGTT -3'	168	MG_251832.1

Primer design and gene selection were based on previous studies on dermatophyte virulence genes (Achterman and White, 2012).

Statistical analysis

All experimental data were analyzed using GraphPad Prism software (version 11.0; GraphPad Software Inc., USA). All experiments were performed in triplicate, and the present results were reported as Mean ± SD. Statistical significance was analyzed using ordinary one-way and two-way ANOVA, with the Geisser-Greenhouse correction followed by Tukey's post hoc test. Differences were considered statistically significant at a p-value less than 0.05 ($p < 0.05$).

RESULTS

Yield of *Solanum incanum* fruit extracts

The amount of *S. incanum* fruit extracts differed significantly according to the extraction solvent used (p = 0.0196) as shown in Figure 1. The aqueous extract exhibited the highest extraction yield at 4.74 ± 0.24%, followed by the methanol extract at 4.21 ± 0.12%. In comparison, n-hexane and ethyl acetate extracts yielded lower amounts, at 3.39 ± 0.19% and 3.19 ± 0.09%, respectively (Table 2).

Total flavonoids, alkaloids, and phenolic contents estimation

The quantitative phytochemical analysis of *S. incanum* fruit extracts demonstrated differences in total flavonoid concentrations (TFC), TAC, and TPC. For TFC, the quercetin standard calibration curve was used, and for TPC, the gallic acid standard calibration curve was used. The quercetin standard calibration curve indicated a linear relationship between quercetin concentration (0-40 µg/mL) and absorbance measured at 510 nm. The regression equation was y = 0.00554x, with a correlation coefficient (R^2) of 0.99659 (Figure 2). The gallic acid standard calibration curve demonstrated the linear relationship between gallic acid concentration (0-50 µg/mL) and absorbance measured at 760 nm. The regression equation was y = 0.00570x + 0.001713, with a correlation coefficient of 0.99990 (Figure 3).

The TAC of *S. incanum* fruit extracts differed according to the extraction solvent used (p = 0.004). The highest alkaloid content was recorded in the hexane fruit extract (HFE) at 2.7 ± 0.1%, followed by the ethyl acetate fruit extract (EAFE) at 2.2 ± 0.1%. The methanolic fruit extract (MFE) demonstrated a lower alkaloid content of 1.4 ± 0.2%, while the aqueous fruit extract (AFE) recorded the lowest value at 1.2 ± 0.4% (Table 3).

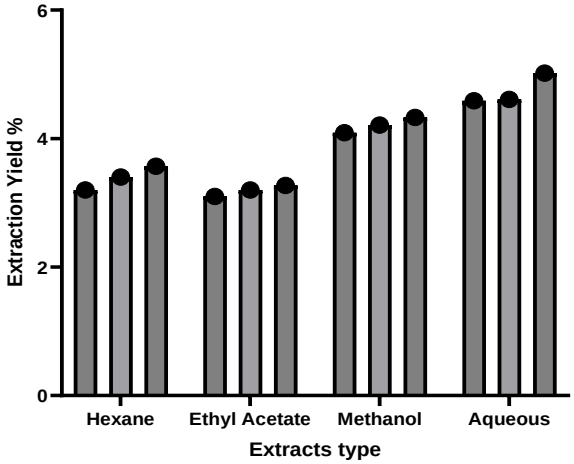

Figure 1. The yields of the *Solanum incanum* fruit extracts from three independent experiments

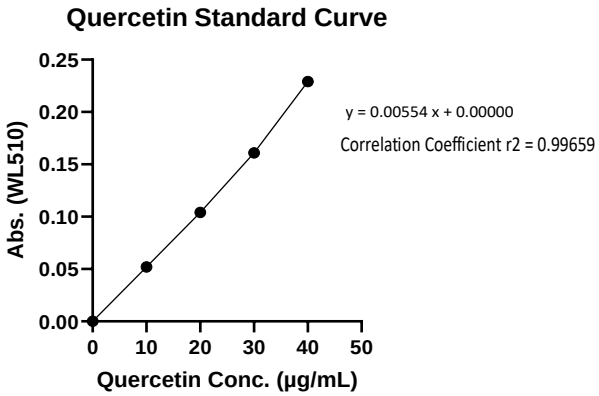

Figure 2. Quercetin standard calibration curve used for total flavonoid content estimation. Abs: Absorbance, Conc: Concentration, WL: Wavelength

Table 2. Extraction yield of the *Solanum incanum* fruit extracts

Extraction solvent	Weight of plant material (g)	Solvent volume	Dry extract (g) Mean ± SD	Yield (%)
n-Hexane	100	1L	3.39 ± 0.19[b]	3.39[b]
Ethyl Acetate	100	1L	3.19 ± 0.09[b]	3.19[b]
Methanol	100	1L	4.21 ± 0.12[a]	4.21[a]
Aqueous	100	1L DW	4.74 ± 0.24[a]	4.74[a]

DW: Distilled water; n-Hexane: Normal hexane. Values are presented as mean ± SD; Measurements were performed in triplicate. There was a statistically significant difference among extraction solvents (p = 0.0196). [ab] Different superscript letters within the same column indicate statistically significant differences at p < 0.05.

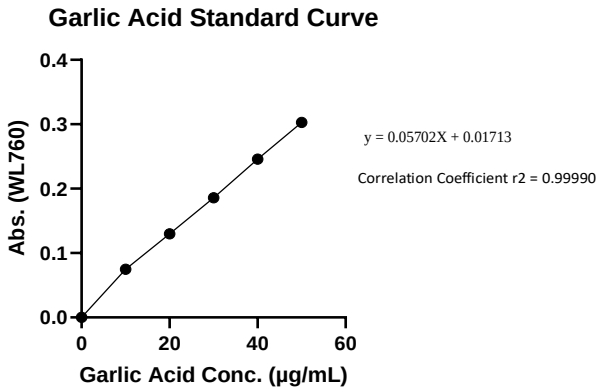

Figure 3. Gallic acid standard calibration curve used for total phenolic content estimation. Abs: Absorbance, Conc: Concentration, WL: Wavelength

Table 3. Total alkaloid content of *Solanum incanum* fruit extracts

Extract Type	Initial extract mass (g)	Total mass (g)	Container mass (g)	Blank residue (g)	Alkaloid mass (g)	TAC (%)
MFE	0.1	1.1024	1.0900	0.0110	0.0014	1.4 ± 0.2^{b}
AFE	0.1	1.1022	1.0900	0.0110	0.0012	1.2 ± 0.4^{b}
EAFE	0.1	1.1032	1.0900	0.0110	0.0022	2.2 ± 0.1^{a}
HFE	0.1	1.1037	1.0900	0.0110	0.0027	2.7 ± 0.1^{a}

MFE: Methanol fruit extract, AFE: Aqueous fruit extract, HFE: Hexane fruit extract, EAFE: Ethyl acetate fruit extract, TAC: Total alkaloid content. Extracts sharing the same superscript letter are not significantly different. Superscript a denotes the highest alkaloid content group (HFE and EAFE), while superscript b denotes the lower alkaloid content group (MFE and AFE). [a,b] Mean different superscript letters within the same column indicate statistically significant differences among extraction solvents ($p = 0.004$).

Phytochemical content of *Solanum incanum* fruit extracts across different solvent extraction methods

The phytochemical content of *S. incanum* fruit extracts differed significantly depending on the extraction solvent. The EAFE and HFE extracts exhibited significantly higher TFC and TAC levels compared to MFE and AFE extracts ($p = 0.0035$). In contrast, the MFE and AFE extracts demonstrated significantly higher TPC than the EAFE and HFE extracts ($p = 0.0035$; Table 4). No significant differences were observed between MFE and AFE, or between EAFE and HFE, for each parameter ($p = 0.193$).

Table 4. Total flavonoids, alkaloids, and phenolic contents of *Solanum incanum* fruit extracts

Type of extract	TFC (mg QE/g extract)	TAC (%)	TPC (mg GAE/g extract)
MFE	1.72 ± 0.24^{b}	1.4 ± 0.20^{b}	19.65 ± 0.30^{a}
AFE	1.88 ± 0.07^{b}	1.2 ± 0.40^{b}	19.39 ± 0.09^{a}
EAFE	3.03 ± 0.07^{a}	2.2 ± 0.12^{a}	2.52 ± 0.03^{b}
HFE	3.43 ± 0.44^{a}	2.7 ± 0.11^{a}	2.58 ± 0.05^{b}

TFC: Total flavonoid content, expressed as mg quercetin equivalent per gram of extract (mg QE/g extract). TPC: Total phenolic content, expressed as mg gallic acid equivalent per gram of extract (mg GAE/g extract). TAC: Total alkannin content. Values are presented as mean ± SD; measurements were performed in triplicate. Comparisons were made within each column (TFC, TAC, and TPC) across different extracts. [a,b] Mean different superscript letters within the same column indicate statistically significant differences at $p < 0.05$.

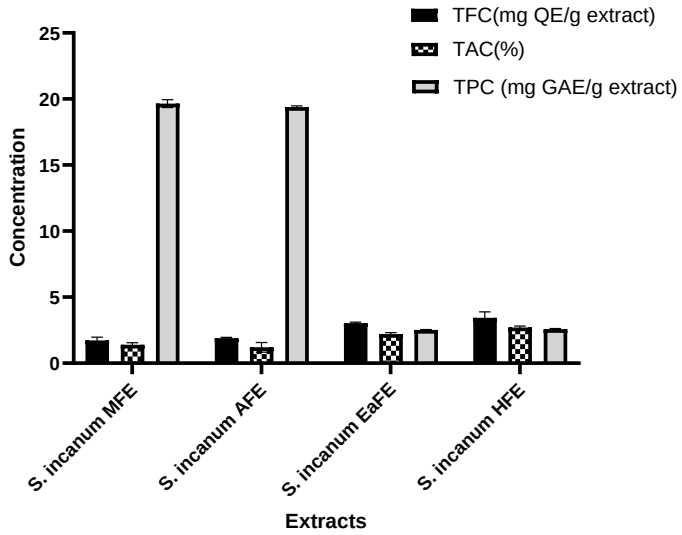

Figure 4. Flavonoid, alkaloid, and phenolic contents of four *Solanum incanum* fruit extracts. TFC: Total flavonoid content (mg QE/g extract), TAC: Total alkaloid content (%), TPC: Total phenolic content (mg GAE/g extract). MFE: Methanol fruit extract, AFE: Aqueous fruit extract, HFE: Hexane fruit extract, EAFE: Ethyl acetate fruit extract.

***In vitro* antifungal activity of *Solanum incanum* fruit extracts**

The MFE exhibited the highest inhibition zone of 14.43 ± 0.17 mm at a concentration of 5 mg/mL (equivalent to 50 µg/disc), followed by the AFE with an inhibition zone of 8.57 ± 0.18 mm. The HFE and EAFE demonstrated significantly lower inhibition zones of 4.54 ± 0.24 mm and 3.96 ± 0.25 mm, respectively ($p = 0.0033$; Table 5). The positive control, terbinafine, demonstrated the highest antifungal activity (15.02 ± 0.10 mm) at a lower concentration of 0.1 mg/mL (1 µg/disc). No inhibition zone was observed with 1% DMSO, confirming its lack of antifungal activity. All treatments differed significantly from one another ($p = 0.0033$; Table 5).

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Table 5. Inhibition zone of *Solanum incanum* fruit extracts against *Trichophyton mentagrophytes*

Treatment	Concentration	Inhibition zone (mm)			
		T1	T2	T3	Mean ± SD
MFE	5 mg/ml	14.23	14.49	14.57	14.43 ± 0.17^{a}
AFE	5 mg/mL	8.38	8.61	8.74	8.57 ± 0.18^{b}
HFE	5 mg/mL	4.32	4.50	4.80	4.54 ± 0.24^{c}
EAFE	5 mg/mL	3.76	3.88	4.24	3.96 ± 0.25^{d}
Terbinafine	0.1 mg /mL	14.92	15.02	15.12	15.02 ± 0.1^{e}
DMSO 1%	--	0	0	0	0^{f}

T1, T2, and T3 represent three independent treatments for each extract. MFE: Methanol fruit extract, AFE: Aqueous fruit extract, HFE: Hexane fruit extract, EAFE: Ethyl acetate fruit extract. DMSO 1%: Dimethyl sulfoxide used at a final concentration of 1% (v/v) as a solvent control. Values are expressed as Mean ± SD (n = 3). ^{a,b,c,d,e,f} Means within the same column followed by different superscript letters are significantly different at $p < 0.05$.

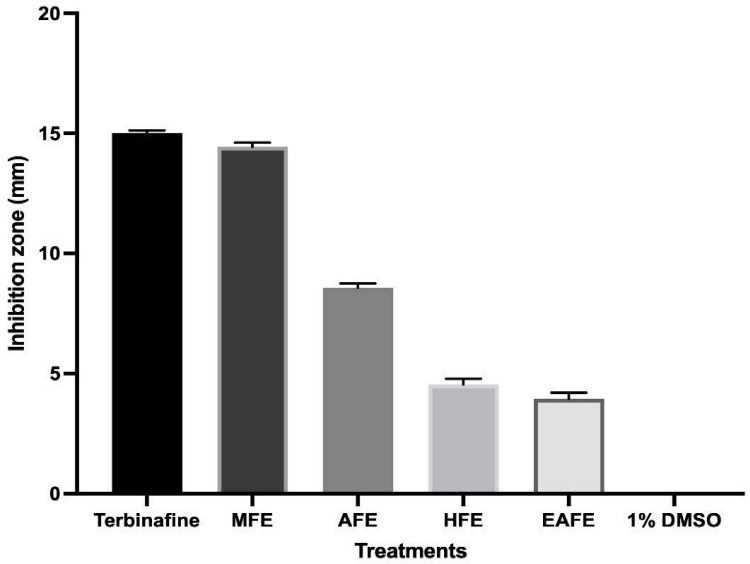

Figure 5. The antifungal activity of the *Solanum incanum* fruit extracts against *Trichophyton mentagrophytes*. MFE: Methanol fruit extract, AFE: Aqueous fruit extract, HFE: Hexane fruit extract, EAFE: Ethyl acetate fruit extract. DMSO 1%: Dimethyl sulfoxide used at a final concentration of 1% (v/v) as a solvent control.

The minimum inhibitory concentration of *Solanum incanum* methanolic and aqueous fruit extracts

The MIC values of *S. incanum* fruit extracts against *T. mentagrophytes* are presented in Table 6. The MFE completely inhibited fungal growth at concentrations ranging from 10 to 0.312 mg/mL, with an MIC of 0.312 mg/mL. The AFE completely inhibited fungal growth at concentrations from 10 to 0.625 mg/mL, corresponding to an MIC of 0.625 mg/mL. The MIC of MFE was one two-fold dilution lower than that of AFE. All triplicates demonstrated consistent MIC values after five days of incubation, indicating complete agreement among them. No visible fungal growth was observed in wells at or above the MIC values, while growth appeared in the control wells and was absent in the sterile control wells. The reference antifungal agent, terbinafine, demonstrated complete inhibition at a concentration of 0.0125 mg/mL.

The minimum fungicidal concentration of *Solanum incanum* methanolic and aqueous fruit extracts

The MFC values of the *S. incanum* fruit extracts are summarized in Table 6. Subculturing from wells exhibiting no visible growth confirmed the complete fungicidal activity of the MFE against. *Trichophyton mentagrophytes* at concentrations ranging from 10 to 0.625 mg/mL, with an MFC of 0.625 mg/mL. The AFE exhibited fungicidal activity between 10 and 1.25 mg/mL, corresponding to an MFC of 1.25 mg/mL. The MFC of MFE was one two-fold dilution lower than that of the AFE, indicating stronger fungicidal potency under the present study conditions. No colony growth was observed on the subculture plates at or above the respective MFC values after five days of incubation. The reference antifungal agent, terbinafine, demonstrated complete fungicidal activity at a much lower concentration (MFC = 0.025 mg/mL). Although both extracts demonstrated fungicidal activity against *T. mentagrophytes*, they were less potent than terbinafine because fungicidal effects were achieved only at higher concentrations. Fungal growth appeared in the growth control plates, but did not occur in the sterile control plates. The current results were consistent across all triplicates.

Table 6. The minimum inhibitory concentration and the minimum fungicidal concentration of *Solanum incanum* methanolic and aqueous fruit extracts after five days of incubation

Extracts	MIC (mg/mL)	MFC (mg/mL)	Ratio
MFE	0.312[b]	0.625[b]	2
AFE	0.625[c]	1.25[c]	2
Terbinafine	0.0125[a]	0.025[a]	2

MFE: Methanol fruit extract, AFE: Aqueous fruit extract. An MFC/MIC ratio <4 indicates a fungicidal effect. [abc] Mean values with different superscript letters within the same column indicate differences in antifungal activity based on MIC and MFC magnitude. Lower values correspond to higher antifungal potency. Results were identical across triplicates.

Expression level of *SUB3* and *StuA*

Expression of *SUB3* and *StuA* was normalized using *GAPDH* and *β-tubulin* as reference genes. Minor differences in Ct values were observed between untreated and treated samples and were presented as Mean ± SD (Table 7). Treatment with MFE and AFE reduced transcript levels compared with the untreated group. For *SUB3*, the Ct value increased from 28.88 in the untreated group to 35.64 in the MFE group and 34.56 in the AFE group, respectively (Table 8). The higher Ct values observed in both treated groups suggested a decrease in transcript level compared to the untreated control, with fold changes of 0.09 for MFE and 0.14 for AFE. Similarly, the expression of *StuA* exhibited an increase in Ct values from 29.08 in the untreated group to 33.50 (MFE) and 32.14 (AFE), with corresponding calculated fold changes of 0.48 and 0.86, respectively. Statistical analysis of ΔCt values revealed a significant difference among treatment groups ($p = 0.0002$). The current results demonstrated that MFE and AFE treatments differed significantly from the untreated group ($p < 0.05$), with MFE demonstrating a greater reduction in the gene expression levels of *SUB3* and *StuA* than AFE.

Table 7. Cycle threshold values of the reference genes following treatment with methanolic and aqueous fruit extracts of *Solanum incanum*

Reference genes	Untreated (Ct)	MFE (Ct)	AFE (Ct)
GAPDH	29.21 ± 0.77	32.23 ± 1.04	31.39 ± 0.67
β-tubulin	27.70 ± 1.18	31.32 ± 1.13	31.19 ± 0.79

Untreated: Fungal inoculum without any treatment, MFE: Methanol fruit extract of *Solanum incanum*, AFE: Aqueous fruit extract of *Solanum incanum*. Ct: Cycle threshold (unitless value representing the number of amplification cycles required for the fluorescent signal to cross the detection threshold).

Table 8. Cycle threshold values and *SUB3* and *StuA* gene expression in *Trichophyton mentagrophytes* following treatment with methanolic and aqueous fruit extracts of *Solanum incanum*

Target genes	Untreated (Ct)	MFE (Ct)	AFE (Ct)
SUB3	28.88 ± 0.16[c]	35.64 ± 0.57[a]	34.56 ± 0.51[b]
StuA	29.08 ± 0.13[c]	33.50 ± 0.52[a]	32.14 ±0.34[b]

Ct: Cycle threshold; higher Ct values indicate lower gene expression levels. MFE: Methanol fruit extract, AFE: Aqueous fruit extract. Values are expressed as Mean ± SD (n = 3). [abc]Mean within the same row followed by different superscript letters are significantly different at $p < 0.05$.

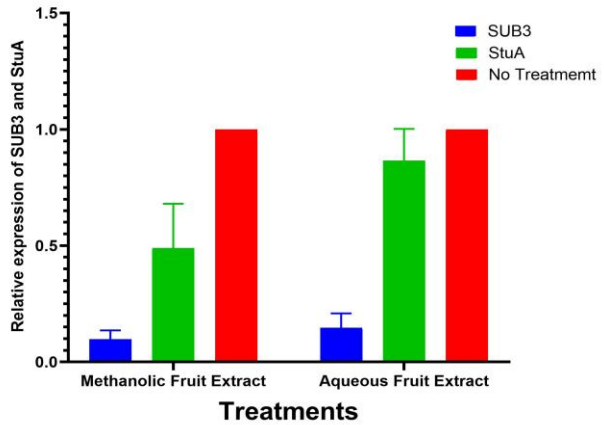

Figure 6. Relative expression of *SUB3* and *StuA* in *Trichophyton mentagrophytes* following treatment with methanolic and aqueous fruit extracts of *Solanum incanum*. Fold change values < 1 indicate downregulation of gene expression.

DISCUSSION

Differences in extraction yield among solvents indicated that polar solvents extracted a higher percentage of hydrophilic compounds. The higher yield from distilled water might be due to its ability to extract polar substances; however, unlike

the sequential solvent fractions, the aqueous extract was obtained from a single extraction. Similar findings have been reported by Do et al. (2014) for medicinal plants, indicating that aqueous and methanol solvents yielded higher extraction amounts due to the abundance of polar secondary metabolites in plant materials.

The results of the present study indicated that solvent polarity played a key role in affecting the efficiency of phytochemical extraction (Azwanida, 2015). Phytochemical quantitative screening indicated that methanol and aqueous extracts possessed elevated concentrations of phenolic compounds, while ethyl acetate and hexane extracts exhibited comparatively larger quantities of flavonoid and alkaloid compounds. The observed pattern demonstrated that plant secondary metabolites exhibit different solubilities across solvent polarities. Phenolic compounds exhibited greater solubility in polar solvents owing to their hydroxyl functional groups, which facilitate interactions with polar extraction solvents (Dai and Mumper, 2010). Several medicinal plants, such as *Azadirachta indica*, *Ocimum sanctum*, and *Moringa oleifera*, indicated solvent-dependent phytochemical differences (Lee et al., 2024; Ranjan, 2024). Methanol extracts often contain higher levels of phenolic compounds, which enhance antibacterial activity (Sasidharan et al., 2011).

The methanolic and aqueous fruit extracts exhibited notable antifungal activity against *T. mentagrophytes*; however, these extracts were evaluated at a considerably higher concentration (50 µg/disc) than the positive control (1 µg/disc), thereby indicating lower relative potency. The discrepancy may be attributed to the fact that crude plant extracts comprise a mixture of compounds with diverse bioactivities, whereas the established medicines consist of a purified compound with a specific antifungal mechanism (Cowan, 1999). The MFE of *S. incanum* had an MIC of 0.312 mg/mL and an MFC of 0.625 mg/mL, whereas the AFE had an MIC of 0.625 mg/mL and an MFC of 1.25 mg/mL. For both extracts (MFE and AFE), the MFC/MIC ratio was 2, which indicated fungicidal activity (MFC/MIC ≤ 4; CLSI, 2008). The current findings demonstrated that *S. incanum* extracts not only inhibited the growth of *T. mentagrophytes* but also eliminated fungal cells. The present study reported an MIC of 0.312 mg/mL for the MFE, indicating notable antifungal activity compared to the previous studies. Several plant extracts, such as *S. hispidum*, demonstrated activity against Trichophyton spp. (Mendoza-León et al., 2022), displaying moderate antifungal efficacy with MIC values of 1 mg/mL (≥1) or higher, depending on variables such as plant species, extraction solvent, and assay conditions. For instance, *Azadirachta indica* leaf extracts exhibited MIC values ranging from 0.5 to 2 mg/mL against *T. rubrum* (Noites et al., 2023). The observed MIC value of 0.312 mg/mL for MFE in the current study indicated comparatively strong antifungal activity compared with other crude plant extracts. While some highly active plant-derived fractions or purified compounds can achieve lower MIC values (< 0.1 mg/mL), this level of potency is generally not observed in crude extracts (Newman and Cragg, 2020). The antifungal efficacy observed in the MFE in the present study aligned with previous findings for taxa within the genus *Solanum*. The methanolic extracts of leaves and fruits of *Artemisia afra* exhibited inhibition zones of approximately 18 mm at a concentration of 50 mg/mL and MIC values ranging from 0.5 to 2 mg/mL against dermatophytes such as *T. rubrum* and *Epidermophyton floccosum* (Afolayan and Meyer, 1997). The differences observed between methanol and the other fruit extracts indicated that solvent-dependent extraction affected antifungal activity against *T. mentagrophytes*, probably due to the larger inhibitory zone recorded in the methanolic extract (Do et al., 2014).

The current findings indicated that treating *T. mentagrophytes* with *S. incanum* fruit extracts reduced the expression of the virulence-related genes (*SUB3* and *StuA*). The methanolic extract caused a greater decrease than the aqueous extract. The notable downregulation of the *SUB3* and *StuA* genes by the MFE corresponded to its antifungal activity observed in MIC/MFC assays. The more pronounced decrease in *SUB3* expression compared to *StuA* may indicate differential sensitivity of these genes to exposure to the extract. Since *SUB3* is linked to extracellular proteolytic activity, which plays a key role in dermatophyte colonization, its suppression could substantially reduce pathogenicity (Bitencourt et al., 2021). The reduced response of *StuA* may indicate a partial, not complete, disruption of the regulatory pathways that control fungal development and virulence. However, transcriptional alterations alone are insufficient to confirm functional suppression of the associated biological processes (Deshpande et al., 2025). The relatively minor alteration observed in *StuA* indicated that not all fungal regulatory genes respond uniformly to the extract treatment. Additionally, upstream signaling pathways such as the cAMP–PKA and MAPK, as well as other transcription factors, might exhibit different sensitivities to the extract (Bitencourt et al., 2021). The selective modulation aligned with the findings of Cushnie and Lamb (2011), indicating that phytochemicals such as flavonoids (quercetin, kaempferol), phenolic acids (gallic acid, caffeic acid), and alkaloids specifically inhibited virulence genes such as *SAP*, *ALS*, and *HWP1* in pathogenic fungi, without impacting their broader regulatory systems. Despite the lack of compound identification in the present study, the observed transcriptional changes suggested that phytochemicals mediated antifungal activity through mechanisms other than direct growth inhibition.

Importantly, the present findings have implications for animal health and veterinary medicine. *Trichophyton mentagrophytes* is a zoonotic pathogen that can be transmitted from animals to humans, particularly among farmers and veterinarians (Plangsiri et al., 2025). The antifungal and gene-modulating effects observed in the present study indicate

that *S. incanum* fruit extracts could serve as a potential alternative or supplement for treating dermatophytosis caused by *T. mentagrophytes* in veterinary practice. The use of plant-based antifungal agents may offer advantages, including affordability, accessibility, and reduced likelihood of resistance development (Gómez-Gaviria et al., 2025).

Despite the promising antifungal activity observed, the potential toxicity of *S. incanum* should be considered. *Solanum incanum* is known to contain bioactive steroidal glycoalkaloids such as solanine and solamargine, which have been associated with toxic effects including gastrointestinal irritation, neurological disturbances, and cytotoxicity at elevated concentrations (Al Sinani and Eltayeb, 2017). These compounds may limit the safe therapeutic use of crude extracts when not properly regulated. Therefore, comprehensive toxicological assessments, such as dose-response studies, safety evaluations, and determination of therapeutic indices, are crucial before clinical or veterinary application. Furthermore, isolating and characterizing specific active compounds can help identify fractions with strong antifungal activity while exhibiting lower toxicity, thereby enhancing the safety profile of potential treatments. The current results of the present study indicated an association between exposure to *S. incanum* extract and altered expression of selected genes linked to fungal pathogenicity, rather than to fungicidal activity alone.

CONCLUSION

The present study demonstrated that solvent polarity significantly influences the extraction yield, phytochemical composition, and antifungal activity of *S. incanum* fruit extracts. Methanol fruit extract demonstrated the highest efficiency and notable activity against *T. mentagrophytes*, along with reduced expression of virulence genes (*SUB3* and *StuA*). Although less potent than terbinafine, *S. incanum* demonstrated potential as an antifungal agent. Nevertheless, the constraints included reliance on a single strain, a limited selection of gene targets, and an *in vitro* study design. Further studies should explore additional species, *in vivo* efficacy, and isolate active compounds to clarify mechanisms and therapeutic potential.

DECLARATIONS

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Authors' contributions

Pita Deta conducted the experiments, collected and analyzed the data, and drafted the manuscript. Peter Ogoti, Daniel Kiboi, and Cristine Bii supervised the study, observed the experiments, and critically reviewed the scientific content of the manuscript. All authors reviewed and approved the final edition of the manuscript before publication.

Availability of data and materials

The datasets produced and examined in the current study are accessible from the corresponding author upon reasonable request.

Competing interests

The authors declared that they have no competing interests.

Ethical considerations

The authors declare that QuillBot was used solely for grammar checking and plagiarism assessment during manuscript preparation. No AI tools were used for data generation, analysis, or preparation of the present study. All scientific content, results, and conclusions were developed and verified by the authors.

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