Clinical Phytoscience

ORIGINAL CONTRIBUTION

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In vitro evaluation of the herbal cream formulation from methanolic leaf extracts of *Tephrosia vogelii* Hook.f for topical application

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Abstract

Background Topical infectious are among of global challenges which propelled up with antimicrobial resistances. This scenario compels for drug discovery and development. Herbal formulation is one of important struggle in the drug development with the purpose of overcoming various diseases distressing health care settings. Thus, this study aimed to formulate and evaluate herbal cream from leaf methanolic extracts of *Tephrosia vogelii* Hook.f for topical therapies.

Methods Herbal cream was prepared by homogeneously mixing up cream base and methanolic leaf extracts of *Tephrosia vogelii.* Physicochemical properties and in vitro antimicrobial activities of the cream were evaluated. The stability, colour change, washability and texture were parameters used evaluate physicochemical properties of cream. Disc diffusion method was employed to evaluate antimicrobial activities of the cream against *Candida albicans* (ATCC 90,028), *Escherichia coli* (ATCC29953) and *Staphylococcus aureus* (ATCC25923).

Results Formulated herbal creams; $CBTV_1$, $CBTV_2$ and $CBTV_3$ with methanolic extracts concentrations content of *Tephrosia vogelii* 0.05%, 0.10% and 0.25%, respectively, were prepared. All cream formulations were stable, washable by water, green in colour and soft. The $CBTV_3$ product exhibited with high prominent antimicrobial activity compared to the rest, henceforth promisingly to be effective for topical skin therapy.

Conclusion The herbal cream formulation of methanolic leaf extract of *Tephrosia vogelii* at 0.25% appeared to have more antimicrobial performance. Therefore, it is considered as minimal concentration for the herbal cream formulation in future for clinical trials as a potential antifungal and antibacterial agent product for treatment of skin infections.

Keywords *Tephrosia vogelii*, Herbal cream, Topical therapy, Skin infection, Disc diffusion

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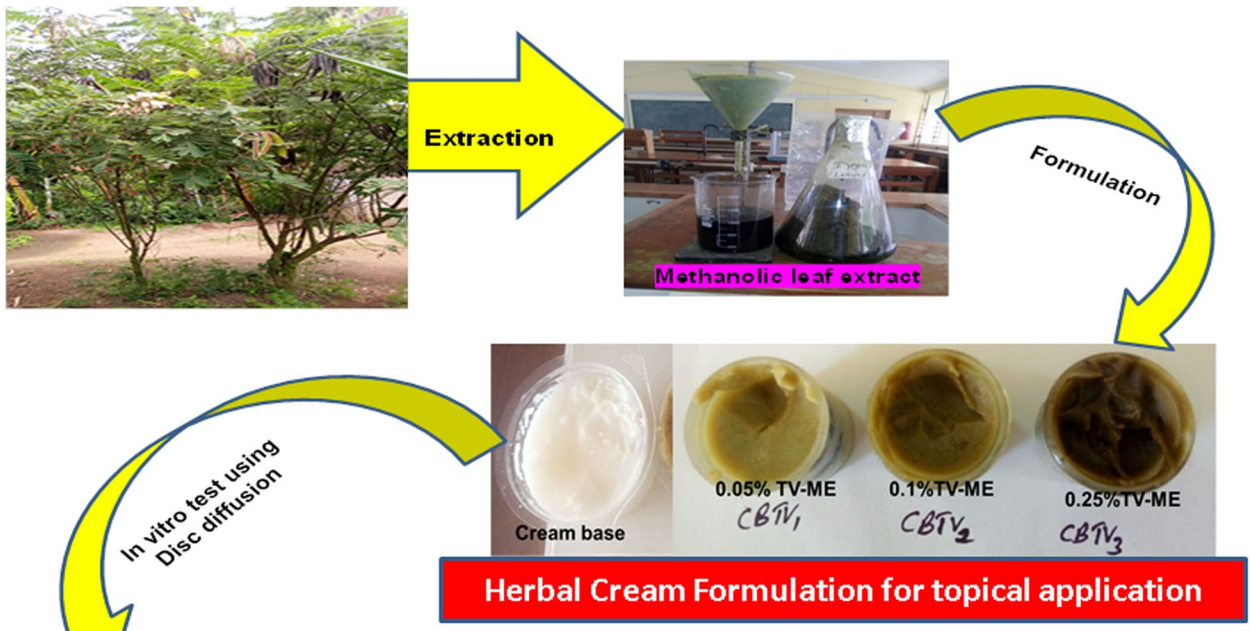
Graphical Abstract



Microbial Species (microorganisms)	Mean Zones of Inhibitions (mm) for Tested Herbal Cream				
	Ciprofloxacin/ Fluconazole (+ve control)	Cream base (-ve control)	$CBTV_1$	$CBTV_2$	$CBTV_3$
Staphylococcus aureus	21.5	0	6.0	8.5	12.5
Escherichia coli	10.5	0	6.0	6.0	6.5
Candida albicans	17.5	0	6.0	7.0	10.5

Introduction

Although often neglected, skin infections significantly pose serious global health challenges. The WHO report and global health studies indicate that skin infections are among of global burden diseases (GBD) as they cause many deaths and fourth greatest causing disability worldwide [1–3]. What still shocking is that the existing petrochemical-derived antimicrobial drugs available on market do not seem to address the problem due to the resistance developed by the microbes to the drugs over time. Such resistance brought challenges to health care practitioners, henceforth, need urgent attention to overcome [4].

Typically, the *Candida albicans*, *Tinea capitus* and *Staphylococcus aureus* are among mostly known for their notoriety in causing topical skin infections [4–6]. Also, these microbes have developed resistance to the conventional antimicrobial drugs to such an extent that the skin infections have become prevalent and intractable in the communities. The *Candida albicans* and *Staphylococcus aureus* are even more dangerous opportunistic and polymicrobial in nature causing serious complicated infections to cure [6, 7]. This indicates that dealing with fungal and bacterial skin infections using the conventional drugs is still challenging. Thus, searching and developing new antimicrobial agents which will offset issues of resistance and decrease cases of skin infections is still a necessary endeavour. At the present time, scientists have paid much attention to search new and/or alternative antimicrobial agents from medicinal plants to cure skin infections. Medicinal plants have been an upcoming and promising deal in drug development against infectious and non-infectious [8–10]. The herbal formulations as prominently practised from Ayurveda in India are one of the means of dosage form to mitigate such skin diseases of human beings [11].

This study systematically investigates the potentiality of *Tephrosia vogelii* Hook.f as a natural resource from which

to search for antimicrobial agents, which could potentially be used to cure fungal skin infections and probably offset drug resistance concerns. This is because there enough evidence that the myriad bioactive compounds reported from this medicinal plant have indicated antimicrobial activities, phytocompounds, and promising inhibitory activities against microbe causing skin infections [12–15]. Phytochemicals such as flavonoids, terpenes and steroids contribute enormously for the antimicrobial activities and medicinal value of this plant [15]. Traditionally, this plant has been used for treatment of bacterial and fungal skin infections for generations and that it could be a potential source of antimicrobial agents development [12]. Such ethnomedical and pharmacological properties of *Tephrosia vogelii* inspires higher steps such as herbal formulation especially semisolids from this plant species.

The semisolids formulations of herbal such as creams, gels and ointments are among important therapeutical forms used for topical dosage therapies [16]. Phytocompounds or extracts as active ingredients from medicinal plants are compounded with semisolid bases to constitute a drug dose for topical application against skin infections. Nevertheless, herbal formulations are an indispensable step followed by antimicrobial activities of either extracts or pure compounds from medicinal plants. Currently, the herbal medicine formulations using native medicinal plants have become common in countries such as China and India, which they employ and market the herbal formulations (semisolids) for addressing skin infection problems in health settings worldwide [17–19]. The herbal medicine formulations from *Tephrosia vogelii*, a medicinal plant predominantly found in sub-Saharan Africa region, have never been reported. Thus, it is in this vein that regarding the reported medicinal properties and pharmacological studies of *Tephrosia vogelii* this study intends to formulate herbal creams from leaf methanolic extracts of *Tephrosia vogelii* found in Tanzania for topical applications.

Material and methods

Plant materials and extraction

The leaves of *Tephrosia vogelii* were collected from Hai district, Moshi region; Northern part in Tanzania (Latitude S 03° 15′ 6.4" and longitude E 37° 14′ 3.8"). The plant species was identified by the plant taxonomist and botanist, Mr. E. John, from Tanzania Pesticides Research Institute (TPRI), and the voucher specimen (SH-NM102) was deposited at the Nelson Mandela African Institution of Science and Technology for future reference. The plant materials were air-dried under the shade for four weeks then pulverized to powder. The pulverized leaves (0.6 kg) were soaked in methanol for 48 h followed by filtration to obtain methanolic filtrates. Methanol solvent was completely evaporated using a rotary evaporator under low pressure below 40 °C. The evaporation afforded 12.0 g of methanolic leaf extracts, which is 2% of the obtained extract from the pulverized leaves materials. Then the extracts were stored at 4 °C prior to herbal cream formulations and biological assays.

Formulation of herbal cream

The formulation of herbal cream involved uniform mixing up of the methanolic leaf extracts of *Tephrosia vogelii* with the cream base. The standard method with minor modifications was employed and furnished herbal cream formulations that composed of two phases (oil phase and aqueous phase) which were uniformly blended as previously described [4, 20–22]. The oil phase with its soluble components and water phase with its soluble components were mixed (Table 1). Each phase with its components was mixed and heated independently at temperature of about 75 °C before mixing up so as to obtain stable mixtures suitable for the storage. The mixture obtained was stirred while hot until cooled to ensure homogeneously composed of the final formulated herbal cream. Herbal creams were prepared in three categories namely $CBTV_1$, $CBTV_2$, and $CBTV_3$ based to the concentrations of leaf methanolic extracts as made of 5 mg/mL, 10 mg/mL and 25 mg/mL, respectively. The extracts were used as active ingredients and cream base used as vehicle for dermatitis therapies [23].

Physicochemical evaluation

The physicochemical parameters such as colour, pH, homogeneity, washability, solubility and stability were pre-meditated to ensure satisfactory results for the formulated herbal cream (Table 1). The physicochemical properties were evaluated using existing techniques as previously described [24, 25]. The stability of formulated drug was assessed at temperature conditions of 4 °C, 25 °C and 37 °C within 6 weeks.

Table 1 Composition of the herbal cream formulations

Oil phase		Water (aqueous phase)	
S/N	components	S/N	components
1	White soft Paraffin	1	Glycerine
2	Cetostearyl alcohol	2	Methyl Paraben
3	Stearic acid	3	methanolic leaf extracts—0.05, 0.1 & 0.25% (Preparing $CBTV_1$, $CBTV_2$, & $CBTV_3$), respectively
4	Organic coconut oil		
5	Propyl glycol	4	Distilled water

Fungi strains, bacteria strains and sub-culturing

Fungal strains, *Candida albicans* (ATCC 90,028) and bacteria strains: the Gram positive bacteria, *Staphylococcus aureus* (ATCC25923) and the Gram negative bacteria; *Escherichia coli* (ATCC29953) used in this study were generously provided by the School of Pharmacy at Muhimbili University of Health and Allied Sciences (MUHAS). All bioassays were performed in the Microbiology Laboratory at MUHAS. All microbes, fungi and bacteria were sub-cultured onto Mueller Hinton Agar (MHA). The MHA (8.0 g) was suspended in 230 mL of distilled water in 500 mL scotch bottle forming the mixture that was heated at 60 ^{0}C to dissolve the agar completely. Then the suspension was autoclaved at 121 ^{0}C for 15 min. The mixture was left to cool at room temperature; inoculation was conducted onto the cooled growth media. Inoculation of strains was carried out then followed by incubation. The fungi strain inocula were incubated at 37 ^{0}C for 48 h while bacteria inocula were incubated at 37 ^{0}C for 24 h.

Antimicrobial activity evaluation

The Disc diffusion method (Disc diffusion technique) as previously demonstrated by Hudzicki (2009) was employed for the antimicrobial bioassay [26]. Antimicrobial assay was conducted to investigate the effectiveness of leaf methanolic extracts of *T. vogelii* contained in the cream formulations. Three cream formulations (25 mg $CBTV_1$, 50 mg $CBTV_2$ and 125 mg $CBTV_3$) and cream base (5 mg CB) contained in separate vials were dissolved in 5 mL of distilled water containing 5% DMSO, hence constituting concentrations of 5 mg/mL $CBTV_1$, 10 mg/mL $CBTV_2$, and 25 mg/mL $CBTV_3$. Discs of 6 mm in diameter were made from the Whatman's no. 1 (filter paper) using a paper puncher. A set of 80 discs placed into storage petri-dish plates, a Muller-Hinton agar (MHA) and Sabouraud dextrose agar (SDA) were autoclaved at 121 °C for 15 min. Then the sterilized MHA) and SDA base plates were seeded with the bacterial and fungal inoculum, respectively with inoculum size 1×10^8 CFU/mL for bacteria and 1×10^7 cell/ mL for yeast. Then 20 µL of each tested cream sample added to sterilized discs, after absorption discs were placed on the seeded agar plates. All sample were placed in one disc plate seeded with one type of species to be tested, each sample was tested in duplicate to minimize results errors. Therefrom was easy to compare effectiveness of three cream formulations ($CBTV_1$, $CBTV_2$, and $CBTV_3$) and cream base (CB) with respective micro-organisms. The preparation was incubated at 37 °C for 24 h to see zones of inhibitions. Zones indicated inhibitory effects of tested samples against pathogens on the cultured plates. Zones of inhibitions were measured with a ruler and compared with the control (commercial pharmaceutical standards) to ascertain the antimicrobial viability. Ciprofloxacin and fluconazole were used as standard drugs against bacteria and fungi, respectively.

Results and discussion

Herbal formulation is a substantial phase towards valuing medicinal plants to combat various diseases in the health settings. In addition to that, herbal cream semisolid formulations are most topically used in the treatment of infected skin. The active ingredients are incorporated in the formulations in order to advance antimicrobial cream, which is applied on the skin to protect it from the microbial skin infections. Certainly, skin care is vital due to its role of protecting the body from external environment messes such as microbial infections. In this study, the physicochemical properties of the herbal cream formulations (Fig. 1) of three different concentrations were evaluated (Table 2). Furthermore, the efficacy of such herbal cream formulation concentrations against *C. albicans, E. coli* and *S. aureus* (Table 3) was studied. The *C.*

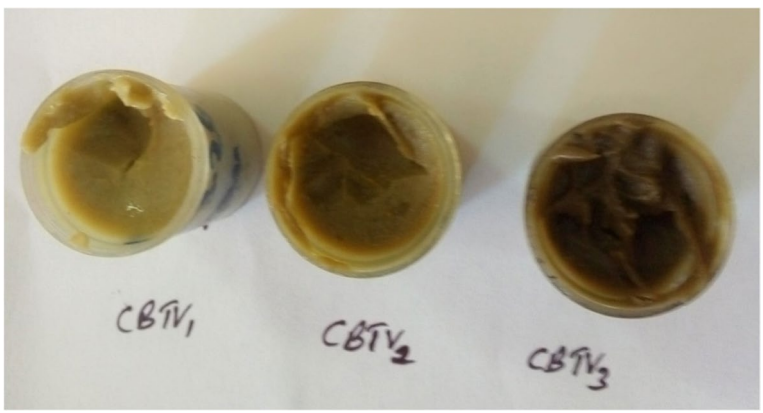


Fig. 1 Herbal Cream Formulations

Table 2 Physicochemical assessment of the herbal cream formulations

Assessed Parameter	Recorded Physical Observation		
	$CBTV_1$ (0.05%)	**$CBTV_2$ (0.10%)**	**$CBTV_3$ (0.25%)**
Colour	Light green	Light green	Deep green
Solubility	Warm water, methanol and ethanol	Warm water, methanol and ethanol	Warm water, methanol and ethanol
Odour	Aroma smell	Aroma smell	Aroma smell
Homogeneity	Homogenous	Homogenous	Homogenous
Washability	water	water	water
Texture	Smooth	Smooth	Smooth
Stability at 4 oC, 25 oC and 37 °C	Stable	Stable	Stable
pH	5.4	5.5	5.7

albicans and *S. aureus* as representative microbes causing topical skin infections [5, 6], were exposed to the cream formulations of different doses.

The effectiveness of bioactives (secondary metabolites) contents contained in the cream formulation was considered in terms of antimicrobial activities that were the measured from the zones of inhibitions. The antimicrobial activities of herbal cream articulated in terms of zones of inhibition ranged from 6.0 to 12.5 mm diameter (Table 3). Correspondingly, zones of inhibition concurs with antimicrobial activities reported in previous studies [13]. Also, the signposted effectiveness of formulated herbal cream might be contributed by bioactives which align with on the reported phytochemicals of the same medicinal plant [15]. The inhibitory performances of the creams appeared to increase with increase in the concentrations of the creams against *Staphylococcus aureus* and *Candida albicans* suggesting that these microbes are more vulnerable to the creams than *Escherichia coli.* All creams have not significantly inhibited *Escherichia coli* probably because the species is resistant to the creams at prepared doses. We anticipate that *Escherichia coli* may be inhibited by creams of higher concentrations of bioactive contents than $CBTV_3$.

As expected, the degree to which the microorganisms responded to the formulated herbal cream samples varied at different doses. For instance, $CBTV_1$ exhibited lowest antimicrobial activity with least zones of inhibitions to all tested microbes. Consistently, $CBTV_2$ exhibited moderate effectiveness as compared to $CBTV_3$. Similarly, $CBTV_3$ and $CBTV_3$ exhibited good activity against both *S. aureus* and *C. albicans* while the *E. coli* demonstrated resistance to all herbal cream doses. The resistance of *Escherichia coli* may significantly be accounted by presence of strong coated cell wall. Overall, these in vitro results clearly indicate that $CBTV_3$ of herbal cream formulation may be a good inhibitory cream since its concentration exhibited the highest antimicrobial activity against microbes reported to cause human skin infections.

Results revealed that the cream base had no antimicrobial effect while standard drugs (ciprofloxacin and fluconazole) exhibited antimicrobial activities. Nevertheless, the antimicrobial activity patterns of herbal cream suggest that the drug (active ingredients) might have both microcidal and microstatic effects [27]. Intuitively, we think that the zones of inhibitions observed over time against the pathogens were attributable to microstatic (bacteriostatic and fungistatic) effects. Accordingly, this study revealed effectiveness of herbal cream with highest concentration within 24 h. We anticipate that the effectiveness might be even more pronounced when dose is repeated especially for in vivo where drug delivery is

Table 3 *In vitro* antimicrobial activity for the tested cream samples

Microbial Species (microorganisms)	Mean Zones of Inhibitions for Tested Herbal Cream				
	Ciprofloxacin/ Fluconazole (+ ve control)	**Cream base (-ve control)**	**$CBTV_1$**	**$CBTV_2$**	**$CBTV_3$**
Staphylococcus aureus	21.5	0	6.0	8.5	12.5
Escherichia coli	16.5	0	6.0	6.0	6.5
Candida albicans	17.5	0	6.0	7.0	10.5

twice a day. This might be concurrent with the ointment formulation from this species that tested in animals [14]. The activeness of the formulated herbal creams resembles with other studies of which the formulation involved methanolic and ethanolic extracts [21, 28, 29]. This indicates that polar solvents are good extractants for potential herbal creams phytocompounds. The performance of herbal cream could be due to the antimicrobial agents contained bioactives such as terpenes, fatty acids, saponins and flavonoids characterized with active moiety and lipophilic features present in as high a concentration as more or equal to 25 mg/mL [30, 31]. We expect therefore that $CBTV_3$ might be effective in treating topical skin disease pathology. However, further study is needed to assess the performance of $CBTV_3$ by in vivo assays using laboratory animal models, which could pave the way to human clinical trials for authenticity therapy delivery.

Conclusion

Human skin is among of vital organs that need high attention to be protected from fungi and bacteria and any microbes causing skin infection diseases. Community is responsible to fight against microbial skin infectious through petrochemical drugs, and the medicinal plants which synthesize voluminous bioactives are best promising alternative natural source against drug resistances that are highly pronounced. Medicinal plants through herbal drugs are the potential focus for drug discovery and development in currently and future. For instance, the performance of formulated herbal cream from methanolic leaf extracts of *Tephrosia vogelii* indicated the capability and potentiality of the plant towards discovery of the antifungal and antibacterial agent that could be used in the treatment of skin infections caused by bacteria *S. aureus* and fungi *C. albicans.* The usefulness of creams was significantly pronounced at the due to the bioactive contents which are predominantly flavonoids, terpenes, tannins and terpenoids. Therefore, this study has substantiated the ability of *Tephrosia vogelii* to serve as a natural resource from which the antimicrobial creams could potentially be discovered and applied to combat skin infections caused by *C. albicans* and *S. aureus*. Formulated herbal creams from *Tephrosia vogelii* revealed to good candidate for drug development, and emphasize more efforts in exploring in medicinal plants to fight diseases in our community.

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Authors' contributions
This work was furnished out in collaboration between both authors. SHM designed the study, wrote the protocol and analysed data under supervision of JAM and MC. Two authors, JAM and MC played same role of guiding and coaching SHM to carry out experiments and participated in writing the manuscript. The author(s) read and approved the final manuscript.

Availability of data and materials
The data used in this study for analysis are available from the corresponding author on reasonable request.

Declarations

Consent for publication
Not applicable.

Competing interests
The Authors declare no competing conflicts of interest.

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