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Caracterización fitoquímica y actividad antimicrobiana de extractos de *Vitellaria paradoxa* (manteca de karité) enriquecidos con aceites esenciales de cáscaras de *Citrus sinensis* y *Citrus limon*

Phytochemical Characterization and Antimicrobial Activity of *Vitellaria paradoxa* (Shea Butter) Extracts Fortified with Essential Oils from *Citrus sinensis* and *Citrus limon* Peels

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Resumen
La manteca de karité (*Vitellaria paradoxa*) es bien conocida por sus aplicaciones cosmecéuticas debido a su alto contenido de compuestos insaponificables. Sin embargo, su olor desagradable puede limitar la aceptación y el uso del producto. En este estudio, se fortificó la manteca de karité con aceites esenciales para enmascarar el olor desagradable y, de esta manera, se examinaron la composición fitoquímica y la actividad antimicrobiana de los extractos de éter de petróleo y de etanol derivados de la manteca de karité fortificada con aceites esenciales de las cáscaras de *Citrus sinensis* y *Citrus limon*. La extracción de la manteca de karité por maceración se realizó con éter de petróleo y etanol, obteniéndose rendimientos de 5.34 ± 0.74 % y 4.11 ± 0.50 %, respectivamente. Los aceites esenciales se prepararon por hidrodestilación, obteniéndose rendimientos de 0.72 ± 0.05 % (*C. sinensis*) y 0.61 ± 0.10 % (*C. limon*). El análisis fitoquímico mostró la presencia de alcaloides, saponinas, esteroides, glucósidos, taninos y terpenoides en los extractos. Se encontró que el D-limoneno era el componente principal de ambos aceites (91.8% en *C. sinensis* y 84.9% en *C. limon*), con algunas cantidades de β-mirceno (2.3–2.5%), β-linalol (0.5–2.1%), terpinen-4-ol (0.1–1.3 %) y endo-borneol (0.8–0.9 %). El análisis antimicrobiano en *Staphylococcus aureus* y *Candida albicans* mostró una eficacia antimicrobiana considerable ($p < 0.05$) en las muestras fortificadas. El extracto de éter de petróleo fortificado con *C. sinensis* mostró el mayor efecto antifúngico contra *Candida albicans*, con zonas de inhibición de 23-42 mm, mientras que el extracto etanólico fortificado con *C. limon* mostró la mayor eficacia antibacteriana contra *S. aureus*, con zonas de inhibición de 42 ± 0.00 mm. Las concentraciones mínimas inhibitorias (CMI) se situaron entre 2 y 50 mg/mL, donde el extracto de éter de petróleo fortificado con *C. sinensis* presentó la CMI más baja (2 mg/mL) tanto para *C. albicans* como para *S. aureus*. Las pruebas realizadas mediante CMB y CMF corroboraron aún más la eficacia de las formulaciones fortificadas con *C. sinensis*. Se puede afirmar que hubo un aumento notable en la eficacia antimicrobiana al fortificar los extractos de manteca de karité con aceites cítricos.

Palabras clave: manteca de karité; aceites esenciales; tamizaje fitoquímico; actividad antimicrobiana; *Staphylococcus aureus*; *Candida albicans*.

Abstract
Shea butter (*Vitellaria paradoxa*) is well known for its cosmeceutical applications due to its high unsaponifiable content. Nevertheless, its unpleasant smell may limit the acceptability and usability of the product. In this study, shea butter is fortified with essential oils to mask the unpleasant smell, and by so doing, the phytochemical composition and antimicrobial activity of petroleum ether and ethanolic extracts of the shea butter that have been fortified with the essential oil of *Citrus sinensis* and *Citrus limon* peel were examined. Shea butter extraction via maceration was carried out in petroleum ether and ethanol, with 5.34 ± 0.74 % and 4.11 ± 0.50 % yields, respectively. The essential oils were prepared by hydrodistillation, resulting in 0.72 ± 0.05 % (*C. sinensis*) and 0.61 ± 0.10 % (*C. limon*). Phytochemical analysis showed the presence of alkaloids, saponins, steroids, glycosides, tannins, and terpenoids in the extracts.

D-Limonene was found to be the major constituent of both oils (91.8 % in *C. sinensis* and 84.9 % in *C. limon*), with some amounts of β-myrcene (2.3–2.5 %), β-linalool (0.5–2.1 %), terpinen-4-ol (0.1–1.3 %), and endo-borneol (0.8–0.9 %). Antimicrobial screening on *Staphylococcus aureus* and *Candida albicans* showed considerable antimicrobial efficacy ($p < 0.05$) in the fortified samples. The petroleum ether extract fortified with *C. sinensis* showed the highest antifungal effect against *Candida albicans*, with inhibition zones measuring 23-42 mm, whereas the ethanolic extract fortified with *C. limon* showed the highest antibacterial efficacy against *S. aureus*, with inhibition zones reaching 42 ± 0.00 mm. Minimum inhibitory concentrations were between 2 and 50 mg/mL, where petroleum ether extract fortified with *C. sinensis* had the least MIC (2 mg/mL) for both *C. albicans* and *S. aureus*. Tests performed through MBC and MFC further corroborated the effectiveness of *C. sinensis*-fortified formulations. It can be stated that there was a remarkable increase in antimicrobial efficacy upon fortifying shea butter extracts with citrus oils.

Keywords: Shea butter; Essential oils; Phytochemical screening; Antimicrobial activity; *Staphylococcus aureus*; *Candida albicans*.

INTRODUCTION

The shea tree (*Vitellaria paradoxa*), a member of the *Sapotaceae* family, is highly valued for the butter extracted from its fruit kernel (Choungo Nguekeng et al., 2021). In Africa, it is widely distributed across several countries, including Togo, Mali, the Ivory Coast, Ghana, Niger, Senegal, Nigeria, and other savannah regions (Nounagnon et al., 2024). Traditionally, shea butter plays a central role in African daily life and medicine, serving both as a cosmetic agent and a therapeutic remedy for conditions such as congestion, rheumatism, and skin infections. It is also consumed as a food ingredient, valued for its nutritional and functional properties (Nounagnon et al., 2024).

Industrial research into the physical and chemical properties of shea butter has expanded its applications, particularly in the cosmetics and personal care sectors, owing to its excellent emollient and medicinal properties (Gyedu-Akoto et al., 2017). Chemically, shea butter is rich in triterpene esters such as tocopherols, cinnamic acid, and phytosterols, along with unsaponifiable compounds including campesterol, stigmasterol, sitosterol, and various triterpenes like cinnamic acid ester, α- and β-amyrin, parkeol, butyrospermol, and lupeol (Zagga et al., 2021). Moreover, it contains phenolic compounds such as gallic acid, catechin, epicatechin, quercetin, and trans-cinnamic acid. Phytochemical analyses have confirmed the presence of diverse bioactive constituents, including saponins, alkaloids, steroids, glycosides, carbohydrates, and tannins (Zagga et al., 2021). While the saponifiable components primarily contribute to the nutritional properties of shea butter, its non-glyceride constituents, such as tocopherols and amyrins, are key to its therapeutic applications (Abdel-Razek et al., 2023).

Shea butter is widely recognized as a potent natural moisturizer with anti-inflammatory, antioxidant, and wound-healing properties (Lin et al., 2017). It is also used for scar reduction, sun protection, and in the treatment of various skin conditions. The antioxidant compounds present in shea butter help to neutralize free radicals, thereby mitigating signs of aging such as fine lines and wrinkles. Its applications further extend to muscle relaxation, lip care, and hair care, underscoring its multifaceted value in natural skincare (Al-Eryani et al., 2021).

Similarly, essential oils from *Citrus* species are known for a wide spectrum of therapeutic activities, including radical scavenging, antimicrobial, anti-inflammatory, antifungal, and wound-healing properties (Caputo et al., 2020; Ameen et al., 2021). The *Citrus* genus, belonging to the *Rutaceae* family, is particularly valued for the aromatic essential oils concentrated in its peels, which represent nearly half the fruit's total mass. These essential oils are rich in phenolic compounds, terpenes, flavonols, and polymethoxylated flavones—compounds that contribute significantly to their biological activity across food, cosmetic, and pharmaceutical industries (Li et al., 2022; Singh et al., 2021).

The growing shift toward natural products in healthcare and cosmetics has spurred intense research into plant-based compounds with the potential to replace synthetic agents, which often have undesirable side effects (Li et al., 2021). Notably, *Citrus sinensis* essential oils are rich in bioactive constituents, including synephrine, hesperidin, polyphenols, limonene, myrcene, α-terpinene, and camphene—compounds with strong antioxidant and antimicrobial activities (Ameen et al., 2021; Singh et al., 2021).

Despite the broad potential of shea butter, its declining marketability, largely due to its unpleasant smell, has prompted interest in fortifying it with natural additives, such as essential oils, to enhance its appeal and functional properties. However, limited empirical data exist regarding the phytochemical and antimicrobial effects of such fortification. Therefore, this study aims to fortify petroleum ether and ethanol extracts of shea butter with essential oils from *Citrus limon* and *Citrus sinensis*, with the objectives of masking its unpleasant smell and enhancing its antimicrobial efficacy.

MATERIALS AND METHODS

Sample Collection and Processing

Freshly processed, unrefined shea butter (*Vitellaria paradoxa*) was purchased from vendors at Fufu Market, Ilorin, Kwara State, Nigeria. The shea butter was produced from locally harvested shea nuts and was obtained in its semi-solid form without further industrial refining or chemical treatment. The samples were transported to the laboratory in sterile air-tight containers and stored at 4 °C until extraction and analysis. Fresh fruits of *Citrus limon* and *Citrus sinensis* were collected from the University of Ilorin farm, Ilorin, Nigeria. The botanical identification and authentication of the plant materials were carried out at the Herbarium of the Department of Plant Biology, University of Ilorin, where voucher specimens were deposited for future reference.

The citrus fruits were manually peeled, and the peels were air-dried under shade at ambient temperature to preserve phytochemical integrity. After drying, the peels were ground into a fine powder using an electric grinder (Model: Qasa QBL-18L40, Qasa Electric, China). The powdered samples were then stored in air-tight, food-grade polypropylene containers (LocknLock, South Korea) at room temperature until further analysis. The microorganisms employed in this experiment were the stock cultures obtained from the Department of Microbiology, University of Ilorin. The bacterial culture, *Staphylococcus aureus*, and yeast culture, *Candida albicans*, were grown and maintained on nutrient agar and Sabouraud dextrose agar slants, respectively, and kept at 4 °C. Before employing them in the experiment, the cultures were sub-cultured and incubated at 37 °C for 24 hours (*Staphylococcus aureus*) and 28 °C for 48 hours (*Candida albicans*).

Extraction Procedure

Successive solvent extraction was employed to fractionate unrefined shea butter (*Vitellaria paradoxa*) into extracts enriched with compounds of differing polarities. Briefly, 500 g of semi-solid shea butter was placed in a 5-L aspirator bottle (Pyrex, USA) and macerated with 1500 mL of petroleum ether (BDH Chemicals, UK) at a sample-to-solvent ratio of 1:3 (w/v). The mixture was maintained at room temperature for 48 h with intermittent agitation to enhance solvent penetration and extraction efficiency. Following maceration, the petroleum ether extract was decanted, filtered through Whatman No. 1 filter paper, and stored in amber glass bottles at 4 °C until further analysis. The residual marc was subsequently exposed to ambient conditions for 6–7 h to ensure complete evaporation of residual petroleum ether. Thereafter, 1500 mL of absolute ethanol (Sigma-Aldrich, USA) was added, and the extraction procedure was repeated under identical conditions. The resulting ethanolic extract was decanted, filtered, and stored in amber bottles under refrigeration until further use. This sequential extraction approach was to enable the recovery of both non-polar and relatively polar phytochemical constituents present in the shea butter.

For essential oil extraction, 500 g of powdered *Citrus limon* and *Citrus sinensis* peels were subjected to hydrodistillation using a Clevenger-type apparatus (BioQuip, USA) for 3 h., following the method described by Lu et al. (2019) with reduced reagent volumes used and triplicate determinations to ensure reproducibility. The collected essential oils were dried over anhydrous sodium sulfate (Merck, Germany), transferred into amber vials, and stored at –18 °C until chemical characterization and fortification experiments.

Screening of the Phytochemical Shea Butter

Extracts

The phytochemical screening was carried out according to Abdelrahim and Kamal Eldin (2016), with modifications involving reduced reagent volumes. The extracts were screened for alkaloids, glycosides, saponins, steroids, tannins, flavonoids, and terpenoids using standard qualitative assays. All reagents used were of analytical grade, and the assays were performed in triplicate to ensure reliability and reproducibility of the results. The qualitative presence or absence of each phytochemical group was recorded based on characteristic color changes or precipitate formation, as outlined in established phytochemical protocols.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Essential Oils from *Citrus sinensis* and *Citrus limon* Peels

The chemical composition of the essential oils extracted from *Citrus sinensis* and *Citrus limon* peels was analyzed using an Orion Micro Mat 412 double-focusing gas chromatography system (Orion Instruments, USA) equipped with a flame ionization detector (FID) and two capillary columns coated with Cp-Sil 5 and Cp-Sil 19 (fused silica, 25 m × 0.25 mm, 0.15 µm film thickness). Samples were injected at a split ratio of 10:1 with an injection volume of 0.2 mL. The oven temperature was programmed to increase from 60 °C to 260 °C at 2 °C/min. Hydrogen gas was used as the carrier at a constant flow rate. The injector and detector temperatures were maintained at 250 °C and 260 °C, respectively. Chromatographic data were recorded by electronic integration of FID peak areas, yielding qualitative profiles without the application of correction factors. Compound identification was achieved through comparison of retention indices and mass spectra with National Institute of Standards and Technology (NIST) mass spectral library data, and published literature values (Ameen et al., 2021).

Fortification of Extracts with Essential Oils

The petroleum ether and ethanol extracts of shea butter were separately fortified with essential oils from *Citrus limon* and *Citrus sinensis*. Fortification was carried out by blending each extract with the respective essential oil at a 2:1 (extract-to-essential oil, v/v) ratio under continuous stirring at room temperature until a homogeneous mixture was obtained. The selection of this ratio was based on previous reports on the antioxidant properties of the essential oils (Ameen et al., 2021), which demonstrated their bioactivity at relatively low incorporation levels. This ensured effective fortification while maintaining the physicochemical stability of the shea butter extracts.

Antimicrobial Property of Shea Butter Extracts Fortified with Essential Oils from Citrus Species

Source and Maintenance of Organisms

Both *Staphylococcus aureus* and *Candida albicans* used as test organisms in this study were obtained from the Department of Microbiology's stock culture collection at the University of Ilorin, Ilorin, Nigeria. *Staphylococcus aureus* was selected for its clinical relevance as a major cause of skin and soft tissue infections and for its increasing antibiotic resistance, which makes it a key target for antimicrobial investigations. Similarly, *Candida albicans* was included because of its importance as an opportunistic fungal pathogen responsible for a wide range of infections, particularly in immunocompromised individuals. The organisms were maintained on nutrient agar (*S. aureus*) and potato dextrose agar (*C. albicans*) slants and stored at 4 °C. To ensure viability and purity, the cultures were periodically sub-cultured onto fresh agar slants and broth media at defined intervals. Prior to each experiment, the inocula were standardized according to McFarland turbidity standards to ensure uniform microbial density across assays.

Reconstitution and Preparation of Stock Concentration

Citrus sinensis and *Citrus limon* essential oils were used to prepare stock from petroleum ether and ethanol extracts of shea butter. Each extract was weighed and dissolved in the known volume of *Citrus sinensis* and *Citrus limon*, i.e., 2 grams of sample dissolved in 10 mL of solvent, to obtain the general stock at 200 mg/mL. Varying concentrations were prepared from the main stock in the following proportions: 200, 100, 50, 25, 12.5, 6.25, and 2 mg/mL by diluting in an appropriate volume of *Citrus sinensis* and *Citrus limon*. They were subsequently stored in air-tight bottles after proper agitation, before storage in the refrigerator until further use.

Preparation of Culture Media

All media used were prepared according to manufacturer's instructions and in the following proportions:13 grams of nutrient broth in 1000 mL of sterile distilled water (13 mg/mL), 28 g of nutrient agar in 1000 mL of sterile distilled water (28 mg/mL), 39 g of Potato dextrose agar in 1000 mL of sterile distilled water (39 mg/mL) and 38 g of Mueller Hinton agar in 1000 mL of sterile distilled water (38 mg/ml). To ensure sterility, all media formulations underwent autoclaving at 121°C and 15 psi for 15 min. This standardized sterilization process effectively eliminated any potential contaminants, ensuring the reliability and consistency of the media used in the experimental procedures.

Standardization of Inoculum

The microbial inocula were standardized using the 0.5 McFarland turbidity standard before antimicrobial testing. Fresh cultures of *Staphylococcus aureus* and *Candida albicans* were adjusted to match the standard visually, with verification by spectrophotometric measurement where necessary to ensure uniform cell density. This ensured reproducible inoculum concentrations for all assays.

Determination of Antimicrobial Activity of Extracts using Well Diffusion Assay

The agar well diffused described by Ajijolakewu & Awarun (2015) was adopted for this test. Using this technique, 0.1 mL of each standardized inoculum (0.5 McFarland turbidity standard equals 1.0×10^8 cfu/mL) for each test organism was spread with a glass spreader onto a sterile Mueller-Hinton (MH) agar plate to achieve uniform growth. After solidification, the plates were left to dry, and then a sterile cork borer (6.0 mm diameter) was applied aseptically to bore wells in the agar plates. For each extract, wells were bored at a number of intended concentrations, as well as one additional well for a negative control. Then 0.3 mL of each extract concentration was added to the wells made on agar plates as above, and an additional well contained the same volume of *Citrus sinensis* and *Citrus limon* as a negative control. The plates were left on the work bench for one hour for the extracts to diffuse into the medium before incubation at 37°C for 24 h. Streptomycin was used as a positive control.

Antimicrobial activity of the extracts was determined after the incubation period and the measurement of zones of inhibition produced by the extracts against the test organisms was taken and compared with streptomycin.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of all extracts against test organisms was carried out using the broth dilution method as described by Ajijolakewu & Awarun (2015). All extracts at concentrations of 200, 100, 50, 25, 12.5, 6.25, and 2 mg/mL were added to 9 mL of sterile MH broth in separate test tubes, yielding a total of 1.0 mL of extracts. Then, 0.1 mL of an 18-h microbial culture was added to each test tube, adjusted to 0.5 McFarland turbidity standard (1.0×10^8 cfu/mL). There were two types of controls: a positive control with plant extracts at different concentrations combined with nutrient broth at the same ratio as in the experiment. On the other hand, the blank control group contained only sterile broth. Following that, the tubes were incubated for a full day at 37°C. Upon observation, the tube with the lowest concentration (highest dilution) that has no detectable growth when compared with the control tubes (using both physical and spectrophotometer observations) was taken as the MIC.

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

Using fresh nutrient agar plates, the MBC and MFC were determined by subculturing the test solution, which showed no discernible growth (no turbidity) at the MIC. To determine the MBC and MFC of the extract required to kill the organism, the plates were incubated for an additional 24 h at 37 °C, following the procedure outlined by Ajijolakewu & Awarun (2015). The test organism(s)' failure to proliferate on the recovery medium (nutrient agar) following incubation indicated the concentration of the bactericidal and fungal agents. It is not considered bactericidal to treat plate(s) that did not show growth at first but did so after additional incubation; these plate(s) are indicative of the bacteriostatic effect of the extracts on the test organism(s).

Statistical analysis

All experiments were carried out in triplicate using OriginPro 2021 (Massachusetts, USA), and values were expressed as mean ± SD. One-way ANOVA test was used to analyze the differences among the means, with a significance level of $p<0.05$.

RESULTS AND DISCUSSION

Extraction Yield of Shea Butter Extracts

Extraction yields of petroleum ether and ethanol extracts of shea butter were determined and shown in Figure 1. The petroleum ether extract yielded 5.34 ± 0.74 % (w/w), whereas the ethanol extract yielded 4.11 ± 0.50 % (w/w). As seen by the greater yield of the petroleum ether extract, this implies that a significant percentage of shea butter extract components were non-polar in nature and hence soluble in non-polar solvents. This could be justified considering the composition of shea butter, which is made up of high percentages of lipids, triterpenes, and phytosterols, among others (Zagga et al., 2021; Abdel-Razek et al., 2023). On the other hand, the relatively low ethanol yield could be attributed to the solvent's selectivity for more polar components.

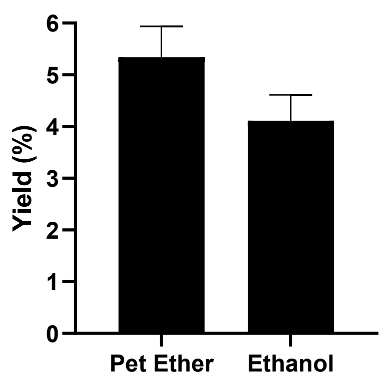

Figure 1. Variations in the yield of petroleum ether and ethanol extracts of shea butter.

Essential Oil Yield from *Citrus limon* and *Citrus sinensis* Peels

The yields of essential oils extracted from *Citrus limon* and *Citrus sinensis* peels are presented in Figure 2. While *Citrus sinensis* yielded a higher amount of essential oil (0.72 ± 0.05 %, w/w), *Citrus limon* yielded 0.61 ± 0.10 % (w/w). Variations in yields can be explained by different features of the plants, such as peel composition, concentration of glands, and other parameters related to both genetics and growing conditions. The same kinds of differences were noted by Li et al. (2022) and Ameen et al. (2021). Based on the results obtained from *C. sinensis*, it can be assumed that it might be used as a source of essential oils for cosmetology.

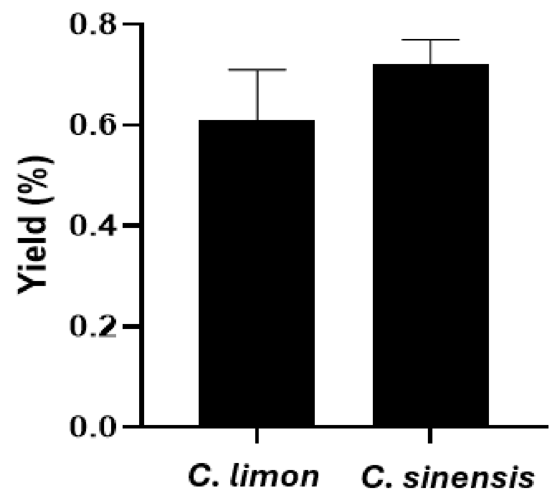

Figure 2. Variations in the yield of essential oil from the peel of *Citrus limon* and *Citrus sinensis*.

Composition of Phytochemicals in Shea Butter Extracts

The phytochemical constituents of petroleum ether and ethanol extracts of shea butter are shown in Table 1. The presence of alkaloids, saponins, and steroids in both types of extracts suggests that the mentioned compounds are abundantly present in shea butter samples. Glycosides and tannins were found only in the ethanol extract, while terpenoids were found only in the petroleum ether extract. On the other hand, there was no presence of flavonoids in either of the two types of extracts.

The variations in the composition of phytochemicals found in both types of extract are attributed to differences in solvent polarity and selectivity for compound classes.

Polar solvents like ethanol have a greater affinity for polar compounds, while non-polar solvents like petroleum ether have better affinity towards non-polar compounds. Similar observations were made by Etubi et al. (2011) and Zagga et al. (2021). Some of the bioactive compounds identified in the extracts may play an important role in the antimicrobial activity observed in this study. The bioactivity profile of the alkaloids includes antimicrobial, antifungal, and anti-inflammatory properties (Dubey & Singh, 2021). Terpenoids are bioactive compounds known to exhibit antibacterial activity by interfering with microbial cell membranes (Guimarães et al., 2019). In turn, tannins are recognized for their ability to act as antimicrobials by interfering with enzymes and precipitating proteins (Demarque et al., 2018). Saponins are bioactive compounds known to have membrane-perturbing activities (Khan et al., 2022).

Table 1. Phytochemicals present in petroleum ether and ethanol extracts of shea butter

Phytochemicals	Petroleum ether	Ethanol
Saponin	+	+
Glycoside	-	+
Alkaloid	+	+
Steroid	+	+
Flavonoid	-	-
Terpenoid	+	-
Tannin	-	+

+ Present; - Absent

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis of Essential Oils of *Citrus limon* and *Citrus sinensis*

The chemical compounds inherent in essential oils extracted from *Citrus limon* and *Citrus sinensis* peels are shown in Table 2. D-limonene was predominantly found in the essential oil of both *C. limon* and *C. sinensis*, representing 84.9 % and 91.8 % of their compositions, respectively. Other important components were β-myrcene, β-linalool, terpinen-4-ol, endo-borneol, α-terpinene, eucalyptol, and β-pinene. The prevalence of D-limonene is consistent with earlier research findings on the chemical composition of citrus peel essential oils (Ameen et al., 2021; Umar & George, 2017). The abundance of monoterpenes and oxygenated monoterpenes present in both oils may contribute to their bioactivities. Ben Hsouna et al. (2017) stated that monoterpenes and their oxygenated analogs contributed largely to the antimicrobial properties of essential oils. Thus, the antimicrobial activity displayed by the fortified extracts may be linked to the presence of D-limonene and other active terpenoids in the oils obtained from citrus fruits.

Antimicrobial Properties of Shea Butter Extracts Containing Citrus Essential Oils

Antimicrobial properties of shea butter extracts fortified with *Citrus limon* and *Citrus sinensis* essential oils towards *Candida albicans* and *Staphylococcus aureus* were shown in Table 3. All formulations were found to possess variable antimicrobial properties depending on the formulation and microorganisms used for testing. Petroleum ether extract containing *C. sinensis* essential oil proved to possess the best antifungal properties since it resulted in inhibition zones of 23 to 42 mm. The second most effective antifungal formulation was ethanol extract fortified with *C. sinensis*, which produced antifungal zones of 21 to 40 mm. Conversely, formulations containing *C. limon* showed lower inhibition zones against the same fungus. The better antifungal properties of the extracts formulated with *C. sinensis* might be attributed to the presence of high amounts of D-limonene and oxygenated monoterpenes in the essential oil of *C. sinensis* (Table 2). These components have been shown to impair the fungal cell membrane and affect cellular processes (Ben Hsouna et al., 2017).

On the other hand, for *S. aureus*, the ethanol extract containing *C. limon* essential oil showed the highest levels of antibacterial activity, providing an inhibition zone of up to 42 mm. The increased level of antibacterial activity in this extract might be explained by the presence of phytochemicals like tannins, glycosides, alkaloids, and saponins, which could synergize well with terpenoid compounds in the essential oil (Table 1). Earlier studies demonstrated that tannins are known for their ability to inhibit enzymes of microorganisms, while alkaloids and saponins can damage cell membranes, contributing to antimicrobial activity (Demarque et al., 2018; Dubey & Singh, 2021; Khan et al., 2022).

Table 2. Predominant compounds in the essential oils from peels of *Citrus limon* and *Citrus sinensis*

Compounds	KI	*C.limon*	*C.sinensis*	MS Data
beta.-Pinene	943	0.7	-	121, 105, **93**, 79, 69
3-Carene	948	-	0.1	121, 105, **93**, 77, 67
beta.-Myrcene	958	2.5	2.3	121, 107, 93, 79, 69
alpha.-Phellandrene	969	0.1	0.1	119, 105, **93**, 77, 65
cis-.beta.-Ocimene	976	0.3	0.1	121, 105, **93**, 79, 67
.beta.-Terpinen	993	-	0.2	121, 105, **93**, 77, 69
alpha.-Terpinene	998	1.3	-	**121**, 105, 93, 77, 65
n-Caprylaldehyde	1005	-	0.1	100, 84, 81, **57**
D-Limonene	1018	**84.9**	**91.8**	107, 93, 79, **68**, 53
o-Cymene	1042	0.4	-	134, **119**, 103, 91, 77
Eucalyptol	1059	0.2	0.6	108, 93, 81, 69, **43**
beta.-Linalool	1082	0.5	2.1	107, 93, **71**, 69, 55
n-Nonaldehyde	1104	0.1	0.1	114, 98, 95, 70, **57**
Terpinen-4-ol	1137	1.3	0.1	111, 93, **71**, 69, 43
endo-Borneol	1138	0.8	0.9	107, 93, 81, **59**, 43
alpha.-Terpineolene	1143	-	0.1	136, **121**, 93, 77, 65
p-Tolylmethylcarbinol	1169	0.1	-	123, 109, 84, 83, **69**
beta.-Citronellol	1179		0.4	110, 95, 70, 57, **43**
Decanal	1204	0.3	-	121, 111, 93, 80, **69**
cis-Geraniol	1228	0.1	-	150, **135**, 117, 107, 91
alpha.-Farnesene	1458	0.1	-	120, 105, **93**, 79, 69

When comparing the fortified extracts to their respective controls, there was an indication of increased antimicrobial activities after fortification. The ethanol extracts had lower inhibition zones than the control, while the citrus essential oils alone had moderate inhibition zones. On combining them, however, there was an increase in the size of inhibition zones against the two test organisms. The synergy observed is attributable to the additive or synergic interaction of phytochemicals present in the shea butter extracts and those from citrus essential oils. There have been reports of such synergetic activities where more than one phytochemical acts on the target microbes, leading to higher antimicrobial efficacy (Guimarães et al., 2019).

As a result of this, the antimicrobial activities observed in this study are attributed to phytochemicals present in both the shea butter extract and the citrus essential oil. The phytochemicals detected in the shea butter extracts included alkaloids, saponins, tannins, steroids, and terpenoids. On the other hand, D-limonene, β-myrcene, β-linalool, terpinen-4-ol, and eucalyptol were some of the phytochemicals identified in the citrus essential oils. The increased activities in the fortified extracts indicate that the products can be used as natural antimicrobial agents in cosmetic and pharmaceutical products.

Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Minimum Fungicidal Concentration (MFC) of the Extract Formulations

The MIC, MBC, and MFC values of the extracts fortified with oils and shea butter extracts are presented in Table 4. Among all the fortifications used in this study, the petroleum ether extract fortified with *Citrus sinensis* showed the lowest MIC values against *C. albicans* (2 mg/mL) and *S. aureus* (2 mg/mL), representing the highest antimicrobial effect of the fortified samples tested. Meanwhile, the extract fortified with *C. sinensis* using the ethanol solvent also demonstrated potent antimicrobial activity, with MIC values of 12.5 mg/mL against *C. albicans* and 2 mg/mL against *S. aureus*. The low MIC values found in the samples fortified with *C. sinensis* are indicative of a good inhibitory effect against the microbial species. The increased activity may be attributed to the presence of the majority component, D-limonene, and some oxygenated monoterpenes present in the extract formulation, as revealed by GC–MS analyses (Table 2). The monoterpenes have well-established functions in altering membrane permeability and interfering with essential cellular functions of microorganisms (Ben Hsouna et al., 2017; Ameen et al., 2021).

Furthermore, the findings revealed by the MBC and MFC assays clearly indicated that *C. sinensis*-fortified formulations were better than *C. limon*-fortified formulations in terms of their bactericidal and fungicidal activity. The reduced bactericidal and fungicidal concentrations clearly show that the active ingredients had the potential not only to inhibit the microbial growth but also to destroy the microbial cells. This is possibly due to the synergistic action of terpenoids found in the essential oils and other biologically active compounds, such as alkaloids, tannins, and saponins, found in the shea butter extracts (Dubey & Singh, 2021; Khan et al., 2022).

The result obtained in MIC, MBC, and MFC tests were supported by the results of the agar diffusion antimicrobial test. Also, the data showed that the addition of citrus essential oils to the shea butter extracts significantly improved their antimicrobial activity. Among all tested formulations, the most active in terms of antimicrobial properties was the petroleum ether extract containing the *Citrus sinensis* essential oil, which had MIC equal to 2 mg/mL in relation to *Candida albicans* and *Staphylococcus aureus*. In turn, the ethanolic extract containing *Citrus limon* essential oil had MIC of 6.25 mg/mL in relation to *C. albicans* and 50 mg/mL in relation to *S. aureus*.

CONCLUSIONS

The current study established that extracts of shea butter and citrus peel essential oils contain high concentrations of bioactive compounds that possess antimicrobial properties. Phytochemical screening of shea butter extract in petroleum ether and ethanol solvents resulted in the detection of alkaloids, saponins, steroids, tannins, glycosides, and terpenoids, whereas GC–MS analysis of the essential oils obtained from *Citrus sinensis* and *Citrus limon* identified D-limonene as the primary compound, as well as several other biologically active compounds, such as decanal, α-farnesene, terpinen-4-ol, α-terpineol, and β-caryophyllene. The shea butter-based extracts fortified with essential oils possessed better antimicrobial properties than those observed in the extracts or essential oils themselves. This finding suggests that there is probably a synergy between the phytochemicals contained in shea butter and the terpenoids found in citrus peel essential oils. Formulations with *C. sinensis* oil demonstrated higher antimicrobial activity. Consequently, it can be concluded that citrus peel essential oils could be efficiently used to fortify shea butter formulations as a means of masking the unpleasant smell of the shea butter and increasing its antimicrobial properties.

Table 3. Antimicrobial activity of pet ether and ethanolic extracts of shea butter fortified with essential oils from peels of *Citrus sinensis* and *Citrus limon* with standard antibiotics against *Candida albicans* and *Staphylococcus aureus*.

	Inhibition Zone (mm)																											
Extracts	**Ethanolic Extract + *Citrus sinensis***							**Ethanolic Extract + *Citrus limon***							**Pet ether Extract + *Citrus sinensis***							**Pet ether Extract + *Citrus limon***						
Concentration (mg/ml)	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**2**	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**2**	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**2**	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**2**
Organism	***Candida albicans***																											
Zones of Inhibition (mm)	21 ± 0.58	29 ± 0.32	26 ± 1.00	35 ± 0.00	40 ± 2.08	38 ± 2.00	34 ± 0.54	12± 1.00	X	14 ± 1.00	13 ± 1.73	18 ± 1.15	18 ± 2.00	10 ± 2.52	23 ± 1.00	30 ± 1.73	28 ± 1.53	35 ± 3.61	40 ± 0.00	42 ± 0.58	39 ± 0.57	X	3 ± 0.00	2 ± 1.00	5 ± 0.00	5 ± 0.23	8 ± 0.01	9 ± 0.00
	Control Ethanolic Extract = 14±0.43mm *Citrus sinensis* = 24±0.82mm							**Control** Ethanolic extract = No zone *Citrus limon* = 11±1.35mm							**Control** Pet ether Extract = 5 ± 0.85mm *Citrus sinensis* = 20 ± 1.12mm							**Control** Pet ether Extract = No Zone *Citrus limon* = 18 ± 0.35mm						
Organism	***Staphylococcus aureus***																											
Zones of Inhibition (mm)	9 ± 1.53	15 ± 0.58	12 ± 0.00	14 ± 1.53	5 ± 1.53	30 ± 0.58	35 ± 2.00	15 ± 2.52	40 ± 3.51	42 ± 0.00	25 ± 4.51	12 ± 0.00	22 ± 4.16	30 ± 3.00	13 ± 0.00	20 ± 2.08	31 ± 5.51	29 ± 0.00	9 ± 4.93	29 ± 6.00	30 ± 6.11	6 ± 0.58	4 ± 1.00	10 ± 1.00	28 ± 0.00	18 ± 3.51	22 ± 3.00	14 ± 0.58
	Control Ethanolic Extract = 10±2.23mm *Citrus sinensis* = 30±0.95mm							**Control** Ethanolic Extract = 4 ± 3.41mm *Citrus limon* = 28 ± 0.67mm							**Control** Pet ether Extract = 12 ± 0.67mm *Citrus sinensis* = 28 ± 2.55mm							**Control** Pet ether Extract = 8 ± 1.28mm *Citrus limon* = 20 ± 0.14mm						

Key: X= No inhibition

Table 4. MIC, MFC, and MBC of pet ether and ethanolic extracts of shea butter fortified with essential oils from peels of *Citrus sinensis* and *Citrus limon* on *Candida albicans* and *Staphylococcus aureus.*

Organism	**MIC (mg/ml)**				**MBC and MFC**			
	Citrus sinensis + Ethanolic Extract	*Citrus sinensis* + Pet ether Extract	*Citrus limon* + Ethanolic Extract	*Citrus limon* + Pet ether Extract	*Citrus sinensis* + Ethanolic Extract	*Citrus sinensis* + Pet ether Extract	*Citrus limon* + Ethanolic Extract	*Citrus limon* + Pet ether Extract
Candida albicans	12.50	2.00	6.25	2.00	+	+	_	_
Staphylococcus aureus	2.00	2.00	50.00	25.00	+	+	_	_

Key: **-** = Static effect + = Cidal effect

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