



Chromatographic and Antidermatophytic Evaluations of Essential Oil Compounds from *Citrus sinensis* Fruit Peel

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ABSTRACT

Essential oils (EOs) are complex natural mixture of chemical constituents, having a major component at a relatively high concentration while the minor ones are present in trace amounts. Hence, the need for a simple and efficient isolation technique required to obtain compound that are bioactive antidermatophytic. Therefore, the study aim to isolate bioactive molecules of EO from fruit peels of Nigerian *Citrus sinensis* (L.) Osbeck with potential antidermatophytic activity against multidrug-resistant tinea capitis dermatophyte. The EO was reaped by hydrodistillation and the bioactive components were examined by thin layer chromatography coupled with direct bioautography assay method. The latter is an assertive technique that targets the isolation of only the active component(s) in a bioassay-guided fashion. The antidermatophytic components were identified with GC-MS as 4-Heptyn-2-ol and Pentane-2, 4- dimethyl. These components (mixture) recorded, are a first-hand report—as present in *Citrus sinensis* (L.) Osbeck fruit peel EO. Our results support a diagnostic method in the isolation of antidermatophytic components from essential oil with an additional confirmation step.

Keywords: Antidermatophytic compounds, thin layer chromatography, direct bioautography, *Citrus sinensis* and essential oil

INTRODUCTION

There is an increased cognizance of resistance to antifungals in the last decade, especially in multidrug resistant dermatophytes causing dermatophytosis (Sonthalia *et al.*, 2021), as well as the report of undesirable side effects caused by these conventional antifungals. These have necessitated researchers to search for new antifungal compounds of plant origin. Plants have always been the mainstay wellspring for new compounds with potential antimicrobial properties in the development of novel chemical prototypes especially in Africa where plants are mainly used in traditional medicine to curb diseases (Yance, 2013).

The Search for new bioactive natural products generally requires a relevant bioassay - guide which would focused on a desired activity such as antifungal (antidermatophytes) as well as various techniques for their isolation and identification (Lopes *et al.*, 2017). Bioassay-guided has been the mainstay in isolation and characterization of bioactive compounds of natural origin (Luo *et al.*, 2020). Although, this method is often time consuming requiring complex steps in the isolation of desired compounds, it is however, still remain a simple technique needed to achieve such goal (Zhang *et al.*, 2017).

Essential oil possess different composition of chemical compounds, but not all these compounds may contribute significantly as antimicrobials (Chouhan *et al.*, 2017). Bioautography enables researchers to identify such

compound/s with antimicrobial activity in a complex mixture such as a crude plant extract. The bioautography technique, couple with TLC is a bioassay (*in situ*) experiment that is frequently used to detect biological activities of bioactive products of natural origin (Agatonovic-Kustrin *et al.*, 2020).

There have been quite some documented studies on the antimicrobial potentials of Citrus species, with most of the investigations focused on the search for bioactive antimicrobial phytochemicals. Nonetheless, this study is of essence in the continued search for effective Phyto-molecules that are of antidermatophytic. Though, the antidermatophytic effect of *Citrus sinensis* (L.) Osbeck essential oil has earlier been investigated using agar dilution method (Ungokore *et al.*, 2021); this paper is however, aimed at the identification of potential antidermatophytic compound(s) from essential oils of *Citrus sinensis* (L.) Osbeck., using the direct bioautography chromatographic methods.

MATERIALS AND METHODS

Identification of antidermatophytic compounds from *Citrus sinensis* fruit peel oil was carried out by applying the bioassay guided principle on TLC-direct bioautography.

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Thin Layer Chromatography (TLC)

Five (5) ml of the EO was dissolved in 1ml of absolute chloroform prepared as an applied solution. Thin layer chromatography was performed with 10 x 20 cm normal particle size silica gel 60 F₂₅₄ aluminum plate. The plates were activated by placing in hot air oven at 100 °C for 10 mins. Two (2) µl of the sample was applied at 1.5 cm above from the bottom using capillary tube micro-pipette on the preconditioned TLC plate. After air drying at room temperature, TLC plate was kept inclined in a TLC chamber (with dimension 25 x 15 x 12 cm). Separation of components of the oil was performed using Hexane and Ethyl acetate at a ratio of 9:1 v/v. The plate was allowed to dry at room temperature for about ten minutes. The plate was then treated with vanillin sulfuric acid reagent and placed in hot air oven at 105°C for 5 minutes so as to view separations on the chromatograms. UV light at 365nm and 254nm were also used to establish the presence of uv active /bioactive spot and as a reference for the bioautography.

Direct Bioautography

The antidermatophytic effect of the EO components was evaluated *in vitro* against a multi – drug resistant clinical fungal isolate (*Trichophyton tonsurans*) earlier reported by Ungokore *et al.* (2021). The dried chromatoplates developed with infusion mode were immersed for 10 s into the fungal cell suspensions (1 x 10⁶ cfu/ml), the excess liquid drained down and the chromatoplate was allowed to dry for two minutes in a laminar flow chamber. The inoculated strip was incubated in a moisture chamber at 28°C then immersed for 10 s in an aqueous solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.1 g MTT in 60 ml distilled water) and incubated at 37 °C for 4 h. The strip was dipped into 70% ethanol for 10 seconds after drying at room temperature.

The image of the bioautogram for inhibition zones of antidermatophyte activity possessing compounds appeared in yellow colour as against blue background when viewed under UV light 254 nm. The TLC strips were placed parallel together as shown in (Plate 1). With the coordination of bioautography strips along and vanillin-stained strip, inhibition zone area was marked with a metal needle on bigger TLC strip (Strip V) under UV light. The located areas were carefully separated with scissors and silica on marked zones was carefully scrapped using a clean scalpel and it was soaked in ethyl acetate for 24 h to elute compounds into solvent in an airtight vial. Then the silica with solvent was centrifuged twice at 10000 rpm, and the collected supernatant was used for GC-MS analysis for identification of eluted compounds

Gas Chromatography Mass Spectroscopy (GC-MS)

The GC-MS analysis of all the sample oils (eluted/isolated) was performed using a Perkin Elmer GC-MS (Perkin Elmer Clarus 680 GC-Clarus SQ 8T MS) equipped with Elite-5 MS

30 m × 0.25 mm × 25 µm capillary column (5% diphenyl, 95% dimethylpolysiloxane). The mass spectrometry detector was an electron ionization system set at 70eV ionization energy. Ultrapure helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. Ion source, mass transfer line, and injector temperature were set at 250°C. The oven temperature was programmed at 60°C for 3°C/min rate and held isothermally for 10 min before finally raised to 250°C at 10°C/min. The oven temperature was programmed at 60°C for 3°C/min rate and held isothermally for 10 min before finally raised to 250°C at 10°C/min. Diluted samples (1/100, v/v in ethanol) of 1 µL were manually injected in the split mode of 120. Mass spectral scan range was at 45–700 m/z, with a solvent delay of 2 min. The components of the extracts were identified based on comparison of their GC relative retention time and their corresponding mass spectra data in a correlational fashion with those of the in build NIST library search of the MS detector machine.

RESULTS AND DISCUSSION

Citrus sinensis (L.) Osbeck fruit peel essential oil subjected to thin layer chromatography -direct bioautography revealed the presence of five compounds (Table 1) with only one being active against dermatophytes spore. The zones of dermatophyte growth inhibition appeared as yellowish clear spot (Plate 1). The isolation of bioactive compound(s) was followed by the characterization of their chemical structure achieved through the use of GC-MS spectrometry. Thus, Figures 3 gives a record of identified compounds from the crude oil extract and bioactive fraction, found to be 4-Heptyne and 2,4-dimethyl-pentane with the concentration of the latter higher than the former (Table 2). The chemical structures of the compound are as shown in Figures 1 and 2.

GC-MS analysis of the bioactive fraction from the crude oil extract of *C. sinensis* fruit peel, ascertained through the bioautographic method, displayed displayed 2 peaks indicating the presence of two (2) compounds, which are oxygenated compound of unsaturated alcohol (4-Heptyn-2-ol) and a saturated hydrocarbon with a composition of 23.86 and 76.14 % respectively. Ogunjinmi *et al.* (2017) reported hexan-1-ol as one of the constituents in *Citrus sinensis* oil. Also Rani *et al.* (2009) reported isolated alkane (a saturated hydrocarbon) from *Citrus sinensis* plant. Our study is also similar with Liarzi *et al.* (2016), who reported 4-Heptyn-2-ol (volatile organic compounds) possess antifungal activity. Wijekoon *et al.* (2013) also reported 3-Heptyne-2, 6-diol, 5, 5-dimethyl as included in the compounds responsible for antimicrobial activity. Qiao *et al.* (2008) and Favela-Hernández *et al.* (2016) reported the isolation of volatile compounds and alkane from *Citrus sinensis* leaves and fruit peel respectively. Antifungal activity of essential oil is governed to a greater extent by volatile oxygenated compounds (Adams and Sparkman, 2007).

Table 1: Bioautographic profile of aqueous oil extract of *Citrus sinensis* against *Trichophyton tonsurans*

Number of spots	Retention factor (R _f)	Bioactive spot
5	0.84	NA
	0.74	NA
	0.53	NA
	0.37	NA
	0.053	Active

NA = Not active

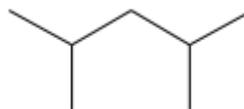
Table 2: GC-MS Analysis of isolated component from bioautographic assay

R.Time	PC(%)	Peak Area (%)	m/z	Mark Name of Compound
7.024	84	23.86	93	4-Heptyn-2-ol
7.570	88	76.14	87	2,4-dimethyl-pentane

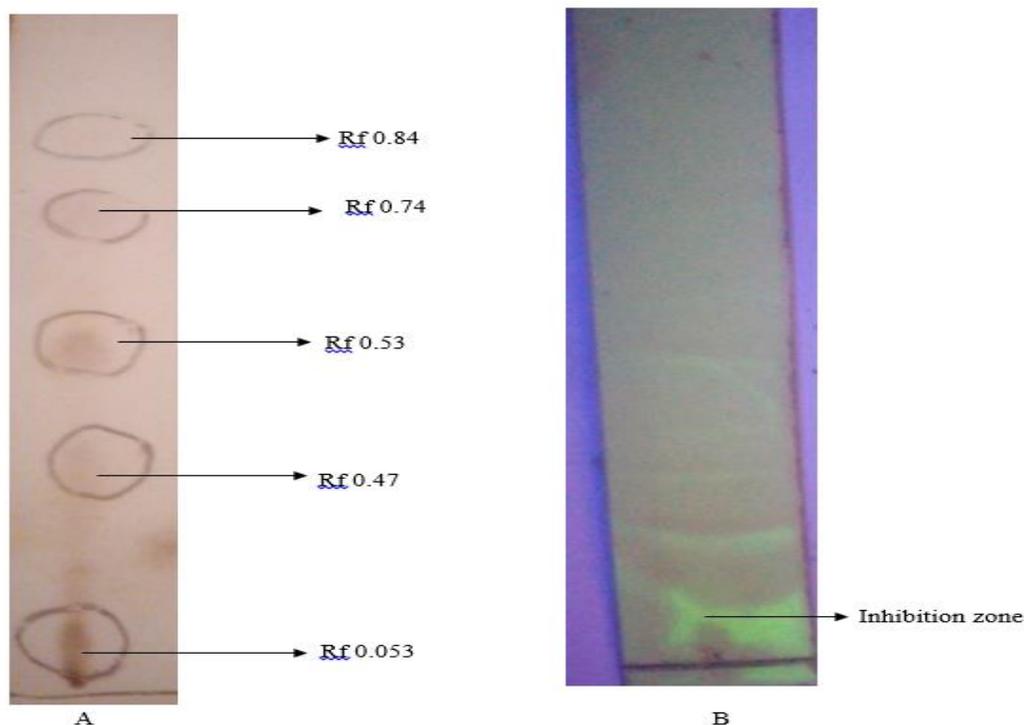
Key: PC = Percentage correlation



4-Heptyn-2-ol



Pentane, 2,4 dimethyl

Figure 1: 4-Heptyn-2-ol**Figure 2 :** 2,4-dimethyl-pentane**Plate 1:** Chromatographic Analysis: **A**—TLC chromatogram of *C. sinensis* EO fraction; **B**—Bioautography assay of the test sample TLC strip on *T. tonsurans*, viewed at 254nm.

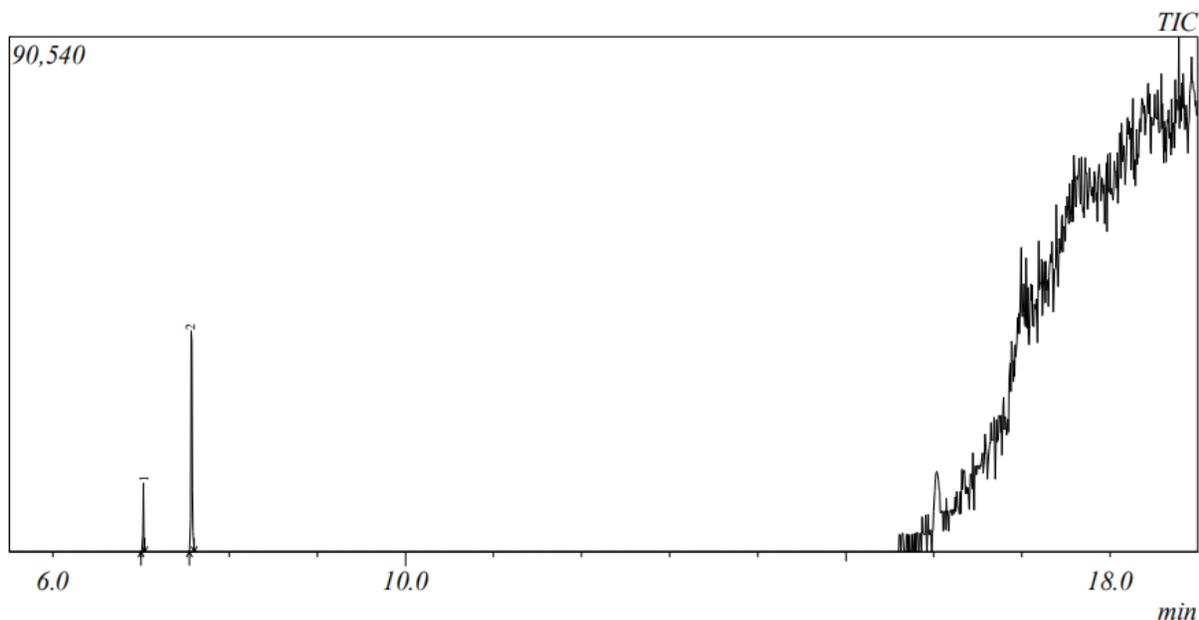


Figure 3: GC chromatogram (RT = 7.024 and 7.570 minutes respectively) of bioactive isolated component (Rf = 0.053) of EO extract from *C. sinensis*, using bioautographic assay

CONCLUSIONS

The study has contributed a valuable diagnostic data for future research in isolation of these antidermatophytic compounds from *Citrus sinensis* fruit peel oil. It has also proven that TLC bioautography serves as a good simple and effective method of investigating natural products (such as essential oil extracts) with acclaimed presence of phytocompounds that have antimicrobial therapeutic indications.

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