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RESEARCH ARTICLE

Comparative analysis of antifungal properties in diverse extracts of *Arcangelisia flava* (L.)

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Abstract

Background: Prolonged fungal infections, specifically those induced by *Candida* species, underscore the critical need to advance efficacious antifungal treatments. **Objective:** The present investigation examines the antifungal characteristics of *A. flava* (L.), emphasising the root extracts of *A. flava*, which contain abundant secondary metabolites such as berberine. **Method:** The preparation of root extracts of *A. flava* utilised solvents of diverse polarities. The antifungal activity of these extracts against *C. albicans, C. glabrata*, and *C. krusei* was evaluated. Determining the Minimum Inhibition Concentration (MIC) and Minimum Fungal Concentration (MFC) for these extracts underscores the significance of selecting an appropriate solvent when extracting bioactive compounds. **Result:** The dichloromethane extract exhibited notable effectiveness, specifically against *C albicans* and *C. krusei*, thus highlighting its potential as a highly potent antifungal substance. To develop targeted therapies and gain a more comprehensive understanding of the antifungal properties of these extracts, it is critical to refine and isolate their active constituents, as demonstrated by this study. **Conclusion:** The findings indicate that A. flava, specifically its dichloromethane extract, may have potential as a reservoir of innovative antifungal compounds. This study makes a valuable contribution to the broader endeavour of incorporating conventional medicinal plants into contemporary pharmacological practices, specifically focusing on their potential to combat fungal infections.

Introduction

The rising prevalence of fungal infections, which can cause significant morbidity and mortality, has created an urgent need for effective therapeutic interventions. The introduction of antifungal agents marked a significant advancement in medical mycology (Garvey & Rowan, 2023). These agents, capable of combating a wide range of fungal pathogens, represented hope in the face of mycotic challenges. However, the demand for novel and potent antifungals has grown as fungi become more resilient and resistance patterns shift (Salmanton-García *et al.*, 2023).

Nature has long been a reservoir of therapeutic solutions due to its vastness. Plants, with their intricate biochemistry, provide a unique blend of phytochemicals used for health benefits across cultures and ages (Shrinet *et al.*, 2021). Many of these phytochemicals have historically exhibited antifungal properties, making plants a valuable source of novel antifungal agents. Traditional medicine's wisdom, combined with modern pharmacological research, has the potential to open up new therapeutic avenues (Anand *et al.*, 2019).

Arcangelisia flava (L.) is a well-known medicinal plant, particularly in parts of Asia. It has traditionally been used to treat a variety of ailments, including fever, hepatitis, and various infections. Because of its widespread use in traditional practices, it warrants further scientific investigation into its therapeutic potential.

Recent research has looked into the pharmacological abilities of Arcangelisia flava (L.). Notably, extracts derived from this plant have been found to have anti-plasmodium properties, highlighting its potential in the

fight against malaria. Furthermore, it exhibits antibacterial efficacy and the ability to modulate cholesterol levels, indicating a broad therapeutic spectrum.

The roots of Arcangelisia flava contribute significantly to its medicinal potential (L.). These roots are high in secondary metabolites, including berberine, an isoquinoline alkaloid known for its diverse bioactivities, and diterpenes. The presence of such metabolites suggests potential antifungal properties, which calls for further investigation.

This current study takes a targeted approach, building on preliminary findings and recognising the critical need for potent antifungals. The authors investigated the antifungal properties of root extracts, determining their Minimum Inhibition Concentration (MIC) and Minimum Fungal Concentration (MFC) against *Candida albicans*, *Candida glabrata*, and *Candida krusei* using solvents of varying polarities.

Methods

Sample collection

The roots of the Arcangelisia flava (Figure 1) were collected in the village of Ranah Sungkai, which is situated in the XIII Koto Kampar sub-district of the Kampar district. Following further investigation, the species identification of A. flava was validated by the Head of the Botany Laboratory in the Department of Biology, the Faculty of Mathematics and Natural Sciences at Universitas Riau.

Figure 1: Roots of *Arcangelisia flava*

Extraction

The extraction process was initiated using the maceration method. Roughly 1 kg of the yellow root sample (*Arcangelisia flava*) was ground into a coarse powder. This powder was then placed in a bottle and submerged with methanol solvent. The sample was consistently shaken every morning and evening for 48 hours, aiming to promote the binding of secondary metabolites within the sample. Following this period, the macerated sample was filtered through cheesecloth and cotton. To ensure thorough extraction of compounds from *A. flava*, the maceration process was repeated 5-6 times or until the macerate appeared colorless. Post-maceration, the solvent was removed using a rotary evaporator, yielding a concentrated methanol extract.

The concentrated methanol extract underwent a partitioning process via liquid-liquid extraction, using a separatory funnel. This method works by segregating components between two non-mixing solvent phases; wherein, some components are soluble in the initial phase and others in the secondary phase. During the process, three solvents, namely *n*-hexane, dichloromethane, and ethyl acetate were employed. The methanol extract was first subjected to n-hexane at a 1:1 ratio, producing an *n*-hexane extract. Subsequent partitioning was done with dichloromethane and ethyl acetate, both maintaining the same ratio. Each resulting extract was evaporated using a rotary evaporator, producing concentrated extracts for each solvent, including n-hexane, dichloromethane, ethyl acetate, and water (Hendra *et al.*, 2022; Hendra *et al.*, 2023).

Antifungal activity assay

Media preparation

Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB) were used in this study to facilitate fungal growth. In particular, 65 g of SDA was suspended in 1 L of distilled water, and 30 g of SDB was suspended in the same amount of water. Both media formulations were sterilised by autoclaving after being heated on a hotplate until boiling.

Fungal

Candida albicans ATCC 10231, C. glabrata ATCC 15126, and *C. krusei ATCC 14243* were cultured on SDA slant agar plates using a cross-streaking method. The revitalised fungal cultures were incubated for 1-2 days at an approximate temperature of 37°C.

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined using a 96-well microplate and a microdilution technique. *C. albicans, C. glabrata*, and *C. krusei* cultures aged 24 hours were diluted in SDB to an optical density (OD) of approximately 0.02 at 530 nm. Using the two-fold dilution technique, various sample concentrations were created. The wells were then filled with 50 µL of media, 10 µL of resazurin, and 10 L of the fungal culture. In addition, positive and negative control wells were also prepared. The microplate was then incubated for 15–20 hours at approximately 37°C. The MIC was visually determined by comparing the color changes in each well to those of negative control.

Minimum Fungicidal Concentration (MFC)

SDA plates were used to determine the Minimum Fungicidal Concentration (MFC). Petri dishes were prepared by pouring 25 milliliters of SDA into them, then allowing the mixture to cool and harden. Ten microliters of solutions with MIC values were plated on the surface of the SDA. Following that, the Petri dishes were placed in an incubator set to 37 °C for 15-20 hours. The MFC was calculated using visual inspection, taking into account the lack of fungal growth and the transparency of the SDA medium.

Results

The study assessed the antifungal efficacy of different Akar kuning extracts against three Candida species: Candida albicans, Candida glabrata, and Candida krusei. The MIC and MFC were measured in parts per million (ppm) (Table I).

For *Candida albicans*, the water extract exhibited a MIC of 250 ppm, but a concentration greater than 1,000 ppm was required to achieve fungicidal effects, indicating moderate antifungal activity but insufficient fungicidal capability. In contrast, the dichloromethane extract inhibited growth at 250 ppm and displayed fungicidal activity at the same concentration, demonstrating its superior efficacy.

For *Candida glabrata*, the water extract required a MIC of 1000 ppm and exhibited an MFC greater than 1000 ppm. This indicates that this yeast strain is relatively insensitive to the water extract. However, the dichloromethane extract was more potent, with a MIC of 500 ppm and an MFC greater than 1000 ppm, indicating that it may contain compounds that are more effective against *C. glabrata*.

Both water and dichloromethane extracts exhibited a MIC of 250 ppm against *Candida krusei*. The MFC for both extracts was 500 ppm, indicating that higher concentrations are necessary for fungicidal activity, although the extracts are effective at inhibiting fungal growth.

Table I: Minimum inhibition concentration (MIC) and Minimum Fungicidal Concentration (MFC) from various extracts of *Arcangelisia flava*

Extracts	*Candida albicans*		*Candida glabrata*		*Candida krusei*	
	MIC (ppm)	MFC (ppm)	MIC (ppm)	MFC (ppm)	MIC (ppm)	MFC (ppm)
Water	250	>1000	1000	>1000	250	500
Dichloromethane	250	250	500	>1000	250	500
Ethyl acetate	250	>1000	1000	>1000	250	>1000
n-hexane	500	>1000	1000	>1000	250	>1000

ppm: parts per million

Discussion

The yield and quality of bioactive compounds obtained are tremendously impacted by the nuances of the secondary metabolite extraction procedure from plant species. The fact that factors such as oxidative degradation, enzymatic activities, and polymerisation processes have the potential to compromise the integrity of compounds present in a plant sample provides support for this claim. In light of this, thisresearch followed the methodology proposed by Yenn et al. (2018) by utilising fresh roots to determine antifungal activity. The justification for selecting fresh plant material is to reduce the likelihood of compound degradation, thus guaranteeing a more precise depiction of the bioactive profile of the plant.

Furthermore, in terms of extraction efficiency and specificity, the choice of an appropriate extraction solvent is not merely a procedural requirement; it is a crucial determinant. As demonstrated by the research of Tong and others, the biological activity of the resulting crude extract is directly impacted by the

nature of the natural products extracted (Tong *et al*., 2014; Martínez-Ávila *et al*., 2021). This research utilised a variety of organic solvents with different polarities for this purpose: dichloromethane, ethyl acetate, and *n*-hexane. The objective of this strategic choice was to maximise the efficiency of extracting lipophilic and hydrophilic molecules, thus guaranteeing a thorough isolation of the bioactive components of the plant (Goryainov *et al*., 2020; Army *et al.*, 2023).

By employing multiple solvents with varying polarities, the extraction process is intended to be more comprehensive and thorough and not merely a matter of convenience. Through the utilisation of solvents spanning from non-polar to polar, it is possible to selectively concentrate on a wide variety of compounds, including those that are hydrophilic or lipophilic in nature. This methodology is critical for optimising the production of bioactive compounds, as it enables the retrieval of an extensive range of secondary metabolites that would otherwise remain unextractable in a solvent system with fewer capabilities (Goryainov *et al.*, 2020; Keddar *et al.*, 2020).

The potent antimicrobial efficacy of the ethanol extract derived from *A. flava* stems has been documented in numerous studies, including those conducted by Setyowati et al. (2014) and Pratama et al. (2018). These studies have observed the extract to be effective against a wide variety of microorganisms, including algae and fungi. Additionally, previous studies have shown that the aqueous extract derived from *A. flava* stems possesses antifungal properties against *Candida albicans*. This is evidenced by a 16.65 mm diameter inhibition zone and a MFC of 40 mg/mL (Setyowati *et al.*, 2014). The extracts' secondary metabolites, which disrupt critical microbial processes such as cell wall formation, protein and nucleic acid synthesis, and metabolic pathways, are primarily responsible for these antimicrobial effects (Cheng *et al.*, 2021).

According to the findings, extracts are selective toward Candida species. The dichloromethane extract had potent antifungal properties. These activities are due to the presence of secondary metabolites in the extract, such as the alkaloid berberine. Berberine, a well-known alkaloid, has a long history of use in Ayurvedic and Chinese traditional medicine. This bioactive compound can be found in many plants, including *Arcangelisia flava*. Various cultures, including Ayurvedic, Chinese, and Middle-Eastern folk medicine, have traditionally used it for its antimicrobial and antiprotozoal properties. Berberine is specifically mentioned in Ayurveda for its significant antimicrobial activity, with preparations like extracts and decoctions being highlighted for their efficacy. The "*Materia Medica*", a classic compendium of Chinese herbal medicine, emphasises the significance of berberine sulfate. This compound has broad-spectrum antimicrobial activity, effectively targeting a variety of microorganisms, most notably Candida species (Imanshahidi & Hosseinzadeh, 2008).

Berberine's pharmacological importance is based on its ability to disrupt critical microbial processes. This includes interfering with pathogens' ability to proliferate and cause disease by interfering with cell wall synthesis, protein production, and nucleic acid replication. The therapeutic application of berberine, however, is dependent on the successful isolation and purification of the active extract (Vuddanda *et al*., 2010; Chu *et al.*, 2014). This procedure is critical for determining the specific components responsible for the antimicrobial properties. This isolation not only aids in understanding the molecular basis of its action, but also paves the way for the development of targeted, more effective berberine-derived therapeutic agents. As a result, ongoing research in this area is critical for improving the authors' understanding and application of this traditional medicinal compound in modern pharmacology.

Conclusion

In this investigation, antifungal efficacy against a variety of Candida species was assessed by preparing extracts of *Arcangelisia flava* according to their polarity. With regard to Candida species, these extracts demonstrated selectivity in their antifungal activity. The dichloromethane extract exhibited the most substantial activity among the aforementioned extracts. In order to gain a comprehensive understanding of and effectively utilise the antifungal properties of *A. flava*, it is imperative to refine and separate the active constituents present in these extracts. In order to ascertain the precise agents accountable for the detected antifungal properties, it is vital to identify and isolate these active compounds. By employing the bioactive components of *Arcangelisia flava*, this procedure will not only elucidate the underlying mechanisms of action, but also facilitate the development of targeted antifungal therapies.

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