PLANT SCIENCE TODAY, 2021
Vol 8(4): 917–921
https://doi.org/10.14719/pst.2021.8.4.1381
ISSN 2348-1900 (online)

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RESEARCH ARTICLE

Evaluation of anti-fungal activity derivative from *Premna odorata* Blanco extract by deep eutectic solvents

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ARTICLE HISTORY
Received: 14 July 2021
Accepted: 18 August 2021
Available online: 28 August 2021

KEYWORDS
Medicinal plants
Antifungal activity
Deep eutectic solvents
Phytochemicals analysis

ABSTRACT

Fungal organisms are an opportunistic pathogen commonly found in different parts in human body like oral cavity and vagina. Recent study has revealed a critical need for novel antifungal medicines developed from medicinal plant extracts due to concerns about fungal pathogen resistance to commercial medication. In the present work, *Premna odorata* Blanco, belonging to the family Lamiaceae was evaluated *in vitro* antifungal activity against two fungal organism isolated from clinical cases. With the aim to replace toxic conventional solvents through deep eutectic solvents was used and phytochemical compounds were determined (total phenol content, total flavonoid content). ChMa extracts of *Premna odorata* Blanco demonstrate a significant antifungal activity against *Candida albicans*, *Monilinia spp*. Higher than water extract. While the DES2 extract reported the highest phenolic contents (3.58 mg GAE/100 g DW) and total flavonoid content (0.028 mg RE/100 g DW) compared with water extract. In conclusion, the study suggests that the *Premna odorata* Blanco extracts by deep eutectic solvents are promising for the development of treatments against various fungal diseases with a friendly green procedure, low toxicity and new application in pharmaceutical industry.

Introduction

The genus *Premna* classified within the Lamiaceae family and subfamily Viticodeae. actually, contains about 200 species which are naturally distributed within Africa, Australia, tropical and subtropical Asia (1). Early studies have reported on the ethnomedicinal values for species of *Premna* in Africa and Asia, notably East and Southeast Asia to treat a wide variety of human body disorders like cough, headache and some other infectious diseases (2, 3). *Premna* is rich in different phytochemical contents like, essential oil, fatty acids, flavonoids and xanthones (4). Many articles have been reported about *Premna* species that have activities against different microorganisms (5, 6). Few study on some types of *Premna* extracts have been evaluated against insects by different methods (4). Fungal diseases are frequent pathogens for human beings, and they may be found in every environment. Candidiasis is the most prevalent fungal disease in humans, and it causes high morbidity among individuals world-wide (7). However, *Candida albicans* is the maximum etiology agent, presenting about 90% of fungal diseases in skin, soft tissue like mouth, vaginal and sometimes the infection progress into systemic infections (8). *Monilinia* spp. are fungal organisms that create economic difficulties in fruits diseases, particularly in post harvest spoilage like brown fruit rot (9).

The conventional antifungal treatments are correlated with many side effects. Moreover, the prolonged use of several synthetic antifungal agents results in various problems, including the emergence of resistant strains and high toxicity (10). In fact, most of the chemical antifungal agents, particularly polyene class and amphotericin B exhibits severe toxicity with fungal resistance, like fluconazole (11). Discovery of new antifungal, low toxic, more effective and affordable drugs is an urgent need. Aromatic plants produce essential oils, which are widely used in traditional medicine. Furthermore, have antifungal activity more than synthetic agents (12). Some bioactive compounds isolated from the medicinal plant, *Gavilea lutea,* have high activity against *Candida albicans* and *Leishmania donovani* (13). In another studies, the essential oils extracted from different medicinal plants showed antifungal activity compared with control as well as in disruption of biofilm formation (10, 14).

Deep eutectic solvents (DES) have received great attention in the field of chemistry as prospective green agents. These mixtures are made up of natural materials that are connected together by hydrogen bonding (15). These mixtures have a big attention in extraction and separation processes due to prominent advantages like: ecofriendly, economic and low toxicity (16). In recent years, DES has been used to extract medicinal plants and bioactive chemicals, which has resulted in improved bioactivity, bioactivity assessments and expression when compared to conventional solvents (17). These steps will lead to its new application in medicinal products industry (18). In the past, no report on the antifungal activity of *Premna odorata* Blanco extracted by deep eutectic solvents has been indicated in the literature. In the current work, for the first time, three types of DES extract of *Premna odorata* Blanco were evaluated against two kinds of fungi microorganism by agar diffusion assay, and also phenolic, flavonoids composition were determined and compared with aqueous extraction.

Materials and Methods

Plant materials

Whole plant of *Premna odorata* Blanco, excluding the roots were collected from the New Plant Variety Protection Testing Centre, Department of Agriculture, Serdang, Selangor, Malaysia and documented in (Institute of Bioscience, Universiti Putra Malaysia) under the respectively voucher numbers MFI 0106/19.

DES preparations

Deep eutectic solvents were synthesized by heating mixture method based on reported studies (7) with 15% of H_2O for all DES kinds. The DESs synthesized are listed in Table 1.

Table 1. Types of DESs used in this study

DES Code	Full Name	M.R
DES1: LGH	Lactic acid, Glucose and Water	5:1
DES2: ChMa	Choline Chloride: Malic acid	1:1
DES3: ChLa	Choline Chloride: laevulinic acid	1:2

Sample extractions

Extraction method was performed based on previous study with some modification (19). The extraction steps were conducted by heating and stirring in a sealed glass container at 40 °C for one hr. The solid ratio 50 mg of dried plants with 1 ml of different DES. The sample was centrifuged at 9000 rpm for 15 min. filtered the suspension through a 0.45 µm nylon membrane prior to work application and analysis. The extraction methods were performed in triplicates in each extract.

Anti-fungal activity

Microorganisms and Growth Conditions

A clinical strain of *Candida albicans* and *Monilinia spp*. isolated from patients referred to Al-Rumadi Teaching Hospital, Alanbar - Iraq. The microorganism was cultured in Sabouraud dextrose agar (SDB) overnight at 36 °C (Himedia, India). The inoculums were prepared by suspension through scraped and diluting the cell mass in sodium chloride 0.9% solution (sterile saline), The suspension was adjusted to 0.5 Mc Farland and measured at 600 nm by spectrophotometer (Apple, Japan).

Antifungal susceptibility testing

The antifungal activity for all extracted was determined by using the disc diffusion method (14) with some modifications. Sabouraud dextrose agar was inoculated with fungi kinds isolated. Sterilized filter paper discs (6 mm of diameter) were soaked in 20 µL of different concentrations of plant extraction (25 mg/ml, 50 mg/ml, 100 mg/ml). Water plant extract and solvent alone kept as control. All inoculated plates and extract paper discs were incubated at 35 ºC for 48 hr. The antifungal activity of plant extracts was determined through growth inhibition zones (mm) by scale and performed in triplicate.

Determination of total phenolic and flavonoid contents

Total phenolic contents (TPC) of *Premna odorata* Blanco extracted by DES were determined according to the procedure (20) with some modifications. In brief, used the Folin-Ciocalteu (F-C) method and the mixture were measured spectrophotometrically at 725 nm. The results based on Gallic acid standard curve and equivalent per 100 g of sample. While total flavonoid content (TFC) of each extract by using aluminium chloride method depended on recent studies with slight modifications (21). The final results gained were equivalent per 100 g of dry mass and expressed on the standard curve of of rutin at various concentrations.

Statistical analysis

Each experiment in current study was performed in triplicate. The mean ± standard deviation (SD) was expressed for results by using SPSS software version 17.0. and Pearson's correlation analysis was done to correlate between results of the phytochemicals and antifungal activity. P-value < 0.05 was set as significant.

Results and Discussion

In the present study, the antifungal screening of *Premna odorata* Blanco extracts against *Candida albicans* and *Monilinia spp* were analyzed through disc diffusion assay. The results presented differences among the three solvents tested for plant extract in various concentrations. Results are shown in Table 2 and Fig. 1.

The highest inhibition result of extract was reported with DES2: CHMA at 100 mg/ml concentration (28.66 ± 1.52 &15.33± 1.51) against *Candida albicans* and *Monilinia spp* respectively when compared with water extract. In contrast, DES3: CHLA of plant extract was achieved the lowest inhibitions results (12.00 ± 1.00 8.66 ± 0.57) against fungi respectively. Whereas, most 25 mg/ml concentration of plant extract in all solvents showed no activity except DES2: CHMA against *Candida albicans* and also

Table 2. Anti-fungal activity by Agar disc diffusion assay

Solvent extracts	Extract concentration mg/ml	*Candida albicans* inhibition (mm)	*Monilinia* inhibition (mm)
DES1	100 mg/ml	24.66 ± 1.52	11.66 ± 1.15
	50 mg/ml	13.33 ± 1.15	10.00 ± 1.73
	25 mg/ml	NA	NA
DES2	100 mg/ml	28.66 ± 1.52	15.33± 1.51
	50 mg/ml	14.66 ± 1.52	11.66 ± 2.00
	25 mg/ml	8.33 ± 1.15	NA
DES3	100 mg/ml	18.00 ± 2.00	12.00 ± 1.00
	50 mg/ml	12.00 ± 1.00	8.66 ± 0.57
	25 mg/ml	NA	NA
Water	100 mg/ml	15.66 ± 1.15	9.66 ± 1.52
	50 mg/ml	8.33 ± 1.52	NA
	25 mg/ml	NA	NA

The values were expressed as the mean ± SD perform in triplicates.

deep eutectic solvents were from 2.51 to 3.58 mg GAE/100 g DW. The highest value of TPC present in DES2 extract was 3.58, followed by DES1extract was

Table 3. Phytochemical screening.

Solvent extracts	TPC mgGAE/100g DW	TFC mgRE/100g DW
DES1	2.92 ± 0.25	0.022 ± 0.005
DES2	3.58 ± 0.14	0.028 ± 0.003
DES3	2.51 ± 0.38	0.027 ± 0.003
Water	1.95 ± 0.32	0.018 ± 0.006

The values were expressed as the mean ± SD perform in triplicates.

2.92 and DES 3 2.51 mg GAE/100 g DW compared with aqueous extract was 1.95 mg GAE/100 g DW. Meanwhile, total flavonoid content values ranged from 0.022 to 0.028 mg RE/100 g DW. The highest value was found by DES2 plant extract, followed by DES3, DES1 respectively. The lowest flavonoid extract for *Premna odorata* Blanco plant was through aqueous extract.

Previous studies performed in some *Premna* plants have identified the presence of phenol, flavonoids, alkaloids and volatile oils when extracted

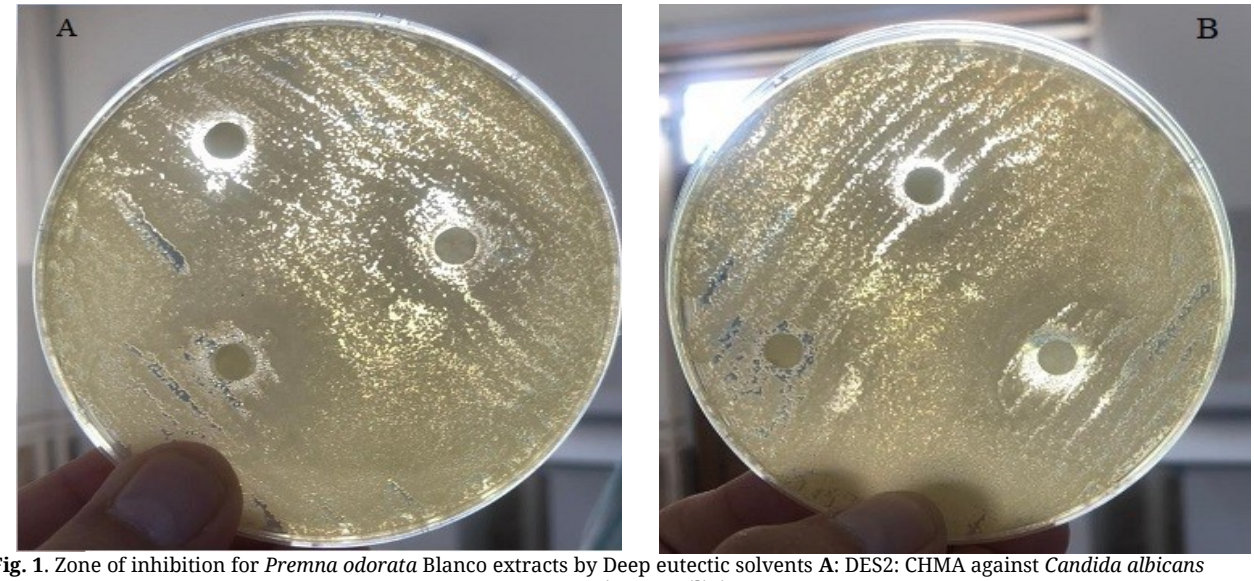

Fig. 1. Zone of inhibition for *Premna odorata* Blanco extracts by Deep eutectic solvents **A**: DES2: CHMA against *Candida albicans* **B:** DES2: CHMA against *Monilinia* spp.

has no inhibition activity against *Monilinia* spp. Based on the results, all the deep eutectic solvents extracts gave various pattern of antifungal activity in some concentrations. Previous studies showed that different kinds of *Premna* genus have antibacterial activity against *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* (22, 23). This is due to the presence of highly bioactive compounds and higher TPC in plants as compared to that of aqueous extract (6). However, only *Premna serratifolia* ethanol extract was found to be active against different kinds of fungi and did not found any antifungal activity by *Premna odorata* extract (24).

Table 3 shows the results for phytochemical analysis content, includes total phenolic content (TPC) and total flavonoid content (TFC). The range value of TPC for *Premna odorata* Blanco extracts by different

by traditional solvents (24, 25). Our group also detected the *Premna odorata* Blanco extract by different types of deep eutectic solvents shows good feedback about phytochemical content in both TPC and TFC compared with water extract, To our knowledge there are no reports to that. Additionally, DES2: ChMa belong to acid-based DESs is polar more than water, this difference in characterization leads to differences in extraction efficiency compared to conventional solvents (18, 26).

In Table 4, Pearson correlation analysis showed very strong correlation between the DIZ values of 2 kinds of fungi and TPC results (R^2=0.981) in DES2 extract, followed by DES1 was (R^2=0.937) a significant ($p < 0.050$). While, moderate correlation with DES3 extract was (R^2=0.656) and water extract was (R^2= 0.327). On the other hand, the correlation was very

Table 4. Correlation coefficients of each analysis

Correlation	DES1	DES2	DES3	Water
TPC versus DIZ	0.937*	0.981*	0.656	0.327
Sig. (2-tailed)	0.026	0.024	0.454	0.788
TFC versus DIZ	0.500	0.866	0.961	0.945
Sig. (2-tailed)	0.667	0.333	0.149	0.178

Note: *Correlation is significant at the 0.05 level (2-taile)

strong between the DIZ values and TFC results (R^2=0.961) in DES3 extract and water. Meanwhile, moderate correlation analysis in DES1 and DES2 (R^2=0.500 & 0.866) respectively.

Conclusion

In conclusion, a plant displays different antifungal activity when extracted by different types of deep eutectic solvents. Of all the three deep eutectic solvents tested, DES2: ChMa was able to extract more bioactive compounds and exhibited strong antifungal activity against two fungi kinds. These solvents will have good future for sustainable and green extraction, for novel application of natural deep eutectic solvents in pharmaceutical products industry. Further researches, including isolation of bioactive compounds from this plant extract by deep eutectic solvents and *in vitro* and *in vivo* assays, in therapy developments for especially fungal diseases.

Acknowledgements

We are thankful to Al Maarif University College (AUC) Alanbar, Iraq for providing and supporting the study.

Authors' contributions

AKA carried out the fungal isolation, identification and editing first draft. ASD prepared DES solvents, plant extraction, activity assay, phytochemical analysis and writing first draft. ROS performed statistical analysis and editing final draft. All authors read and approved the final manuscript.

Conflict of interests

The authors declare that there is no conflict of interest.

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Peer review information: *Plant Science Today* thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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To cite this article: Abdullah A K, Dheyab A S, Saleh R O. Evaluation of anti-fungal activity derivative from *Premna odorata* Blanco extract by deep eutectic solvents. Plant Science Today. 2021;8(4):917–921. https://doi.org/10.14719/pst.2021.8.4.1381

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