Antibacterial activity of some medicinal plants extracted by deep eutectic solvent as a green solvent

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Abstract

Paper's purpose: some medicinal plants have medication history through richly bioactive compounds. In this study, we aimed to evaluation the antibacterial activity and phytochemical contains of these medicinal plants extraction by deep eutectic solvent as a green matter.

Methods: We selected three medicinal plants and extracted by three different kinds of deep eutectic solvent. Three methods (DIZ, MIC Alamar blue assay and MBC) were used to determine the antimicrobial activity. Phytochemical compositions, total Phenols and flavonoid content were estimation by using spectroscopy.

Results: All the medicinal plants used in this study showed a clear different inhibition activity against bacterial strains. the DES2: CHMA for *P. odorata* extract provided the strongest result of antibacterial activity by using MIC and MBC assay against *Staphylococcus aureus*, *Pseudomonas aeriginosa* and *Escherichia coli*. While DES3: CHLA and DES2: CHMA showed the highest amount of phytochemical contains from all plant kinds used more than DES1: LGH.

Conclusions: In this study, all the medicinal plants extracted by DES had various activities and secondary metabolites which may be have activities against bacterial strain.

Keywords: Medicinal plants; Deep eutectic solvent; Antibacterial activities; phytochemical contains

Introduction

Plants have not play a chief role in supplementary value only but also, have a big role in the medicinal plants values (1). The conventional herbal plants have a great contribution in improvement of modern studies for the discovery new drug (2). Many recent drugs were extracted from herbal medicinal plants because have been a major way of treatments for all organisms(3). In the developing countries there are need urgent and rising demand for medicine plants-based because, depended more than 80% population on herbal- based treatment for necessary healthcare (4).

The bacterial pathogenic have strong resistance for chemical antibiotic due to many causes, therefore still continuous searching for potential bioactive compounds from medicinal plants against bacterial diseases (5). Public health problems based on much bacterial infectious disease by *Staphylococcus aureus*, *Salmonella Thypimurium, E.coli* and these bacterial infection have clear clinical finding like acute gastroenteritis, rhinitis, diarrhea and dermatitis (6). Therefore, it is urgent need to discoveries alternatives bioactive compound from plant extracts; in addition the cumulative causes that resulting from chemical treatments (7).

A deep eutectic solvent are usually consist from 2 components and always knows non-toxic mixtures with special properties (8). However, these solvent kinds are prepared easily, are not requiring to high process steps in preparation and ecofriendly properties through easily in biodegradable, low vapor and recyclable (9). All of these hot points that will lead to use the deep eutectic solvents over than traditional solvents in plants extraction procedure (8).

Moreover, traditional medicinal plant extractions have many drawbacks, like low performance, high power used and solvents toxicity that will lead to humanity and ecology effect (10). Therefore, the present study was conducted to evaluate the antibacterial activity against three pathogenic bacteria from some medicinal plants extracted by deep eutectic solvent and its phytochemical analysis

Material and Methods

1. Plant Materials

In this study used a 3 different medicinal plants (*Moringa oleifera* Lam, *Premna odorata* Blanco and *Ocimum basilicum* L.). We used the whole plant but without root for each kind.

2. NADES preparation

All deep eutectic solvents including DES1: lactic acid−glucose (LGH), DES2: choline chloride−malic acid (CHMA) and DES3: choline chloride-lavevulinc acid (CHLA) were prepared by heating method according to related studies (11)

3. Extraction process

The extraction process was performed according to previous studies with some modification (12). The extraction process was performed according to previous studies with some modification. Through used sealed conical flask with 10mg plants to 1ml DES mixture ratio. The mixture was heating gently with stirring on hot plate at 40 °C for 1 h. then transferred the mixture sample to test tube for centrifugation for 10min at 9000 rpm. After that the super tenant was filtered by 0.45µm cellulose acetate filter in triplicate performed.

4. Antibacterial activity methods

4.1 Microorganisms and Growth Conditions:

Three bacterial strain were used in present study, two gram negative (*Escherichia coli and Pseudomonas aeriginosa*) and one bacteria is gram negative (*Staphylococcus aureus*) to estimate the antibacterial susceptibility. These bacterial kinds were obtained from Central Laboratory of Excellence for Advanced Pathological Analysis of Alanbar-Iraq through different clinical samples.

4.2 Agar disk diffusion test:

This method was carried to determine the activity of extracts against various bacteria according to recent study with some modification (13). Through cultured 0.5 Mc Farland standards (1.5 x 10^8 CFU ml) for each bacterial strain in Mueller-Hinton agar plate. Prepared different amount in stock solutions 50 and 100 mg/ml for each plant extract in different DES solvents through dissolved in DMSO. followed by were soaking the filter paper discs (6 mm diameter) in various stock solution amount for adequate time and were dried also. Subsequently, preincubation for 1hour at RT degree. antibacterial activity were measured after overnight incubation period at 37°C, through the zone of inhibition of growth in mm. were applied ampicillin (30µg) as positive control.

4.3 Broth microdilution method:

Minimum Inhibitory Concentration (MIC) outcomes were evaluated by a modified Microplate Alamar Blue (MABA) method (14). In ninety six -well microplates 100 microliters of Mueller Hinton Broth were added. 100 microliters of 50 mg/ml sample stock for each plants extract were added to the wells in first row A in three replicates form. 100 microliters serial transferred from well A till well G to ranged concentration from (50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 mg/ml). One hundred microliters of bacterial cultures suspension 0.5 Mc Farland standards (1.5 x 10^8 CFU ml) was added to all wells that have different extracts concentration. After incubation at 37 _C for 1day was performed with added Alamar Blue reagent and 10% Tween 80 to all well that have extract. For identification microbial growth through this reagent by change the color

(blue color remained mean no bacterial growth and a pink color mean a growth recorded). The Minimum Inhibitory Concentration (MIC) of plants different concentration extract was recorded as the lowest concentration of samples which did not allow a color change. While Minimum Bactericidal Concentration (MBC) value was recorded through wells that remained blue color and transferred about 10 microliters of each well contents on agar plate and incubated for overnight in growth appropriate conditions.

5. Determination total phenolics contents (TPC)

The total phenolics contents of plants extract by DES were determined through used by the Folin-Ciocalteu (F-C) method(15). 1 mL for each plant extracts at (25mg/mL) concentration was added to (5 mL) of F-C reagent solution, vortexes gently and keep for 5 min at room temperature. Later 4 mL of (75g/L) sodium carbonate was added. Mixture was incubated for 30 min at at room temperature. Measured the absorbance at 765 nm after incubated time. The results depended on triplicate analyses and a reference standard used was Gallic acid. The TPC results were expressed as milligram Gallic acid equivalents/ 100g dry weight.

6. Determination total flavonoid content TFC

Total flavonoid content for each plants extract was determined according recent studies with slight modification (16). Specifically, 1ml of plant extracts (25mg/mL) was mixed with 4 ml of distilled deionized water. Then, 0.3 mL of 5% sodium nitrite solution (NaNO2) was added into each test tube. After five minutes, 0.6 ml of 10% aluminium chloride (AlCl3) were added and incubated for one min. Then, 2mL of 1M sodium hydroxide (NaOH) and mixed by vortex. Incubated for fifteen min and the absorbance measured immediately at 510 nm. The TFC results on triplicate were obtained as mg of rutin equivalents/ 100g of dry mass.

7. Statistical Analysis

Each measurement in this study was in triplicate and calculated for the average value. The results obtained from Agar diffusion assay, TPC and TFC were calculated also with ± standard deviation. Statistical analyses were done using SPSS / PASW statistic 18.0 for Windows.

Results

1. Antibacterial activity by Agar disk diffusion assay.

All the medicinal plants used in this study showed a clear different in the diameters of inhibition against used three bacterial strain, that extracted by DES1: LGH, DES2: CHMA and DES3: CHLA in different concentrations. In 100mg/ml concentration showed a range between (17.7-22.7 mm, 17.5-19.7 mm, 23.07-21.7mm) against following bacterial strain *Staphylococcus aureus, Pseudomonas aeriginosa* and *Escherichia coli* respectively.

However, in 50mg/ml concentration also showed inhibition activity range between (15.2-19.8 mm, 14.3-17.1 mm, 16.4-21.9 mm) against bacterial strain in same growth conditions.

The highest result of activity was observed from DES3: CHLA extract of *M. oleifera* (23.07mm and 21.9mm) against *E. coli* at 100 and 50mg/ml concentration respectively. While, showed *P. odorata* extract by DES2: CHMA highest result (22.7mm and 19.8mm) against *Staphylococcus aureus* in different concentrations 100 and 50mg/ml respectively. However, the *O.basilicum* extract by DES2: CHMA observed highest result (19.7mm) against *Staphylococcus aureus* and *E. coli* bacterial strain in different concentrations.

Meanwhile in all plant extracts showed lowest activity by DES1: LGH extract of *M. oleifera* (13.23.mm and 11.53mm) against *Staphylococcus aureus*, *P. odorata* extract by DES1: LGH (14.1mm and11.9mm) against *Pseudomonas aeriginosa* and *O.basilicum* extract by DES1: LGH (12.7mm and 9.3mm) against *Pseudomonas aeriginosa* at 100 and 50mg/ml concentrations respectively. The results are presented in table (Table1).

Table1: Antibacterial activity by Agar disk diffusion assay

Plants Scientific names	**Solvent extracts**	**Extract Concentration mg/ml**	***Staphylococcus aureus***	***Pseudomonas aeriginosa***	***Escherichia coli***
M. oleifera	**LGH**	**100 mg/ml**	**13.2** ± 1.30	**15.1** ± 0.36	**18.4**± 0.25
		50 mg/ml	**11.5** ± 0.15	**14.3** ± 0.26	**16.2** ± 1.20
	CHMA	**100 mg/ml**	**15.3**± 0.30	**17.3**± **0.25**	**21.8**± 0.30
		50 mg/ml	**14.1**±0.20	**16.6**±0.25	**19.7**±0.45
	CHLA	**100 mg/ml**	**17.7**±0.20	**19.4**±0.24	**23.07**±0.73
		50 mg/ml	**15.2**±0.15	**17.1**±1.39	**21.9**±0.36
P. odorata	**LGH**	**100 mg/ml**	**17.3**±0.40	**14.1**±0.36	**19.4**±0.30
		50 mg/ml	**15.1**±0.86	**11.9**±1.26	**16.8**±0.35
	CHMA	**100 mg/ml**	**22.7**±0.30	**19.7**±0.15	**21.7**±0.40
		50 mg/ml	**19.8**±0.40	**16.1**±0.28	**18.9**±0.35
	CHLA	**100 mg/ml**	**21.2**±0.30	**17.3**±0.25	**20.2**±0.21
		50 mg/ml	**19.1**±0.15	**15.2**±0.27	**17.3**±1.32
O.basilicum	**LGH**	**100 mg/ml**	**15.4**±1.00	**12.7**±0.35	**16.2**±0.81
		50 mg/ml	**13.6**±0.55	**9.3**±0.39	**13.9**±0.61
	CHMA	**100 mg/ml**	**19.7**±0.25	**17.5**±0.30	**19.7**±0.49
		50 mg/ml	**16.9**±0.37	**14.3**±0.24	**16.4**±0.56
	CHLA	**100 mg/ml**	**17.3**±1.12	**15.2**±0.55	**17.8**±0.59
		50 mg/ml	**14.8**±2.91	**13.4**±0.60	**14.6**±0.89

Control	Ampicillin (30µg)	**21.9** ± 0.31	**22.3** ± 0.25	**19.1** ± 0.47

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

2. Antibacterial susceptibility test.

Table 2 and table 3 shows the result recorded for the minimum inhibitory concentration and Minimum Bactericidal Concentration (MIC and MBC). The MIC result observed in DES3: CHLA *M. oleifera* extract against *Staphylococcus aureus, Pseudomonas aeriginosa* and *Escherichia col*i were (6.25mg/ml, 3.12mg/ml and 1.56mg/ml) respectively. And DES2: CHMA for *P. odorata* extract against same bacterial strain (3.12mg/ml, 6.25mg/ml and1.56mg/ml respectively. While DES2: CHMA *O.basilicum* extract showed (3.12mg/ml, 12.5mg/ml and 6.25mg/ml) respectively.

Table 2: minimum inhibitory concentration

Plants Scientific names	**Solvent**	***Staphylococcus aureus***	***Pseudomonas aeriginosa***	***Escherichia coli***
M. oleifera	**LGH**	**25** mg/ml	**12.5** mg/ml	**6.25** mg/ml
	CHMA	**6.25** mg/ml	**3.12** mg/ml	**3.12** mg/ml
	CHLA	**6.25** mg/ml	**3.12** mg/ml	**1.56** mg/ml
P. odorata	**LGH**	**6.25** mg/ml	**6.25** mg/ml	**3.12** mg/ml
	CHMA	**3.12** mg/ml	**6.25** mg/ml	**1.56** mg/ml
	CHLA	**6.25** mg/ml	**6.25** mg/ml	**3.12** mg/ml
O.basilicum	**LGH**	**12.5** mg/ml	**25** mg/ml	**12.5** mg/ml
	CHMA	**3.12** mg/ml	**12.5** mg/ml	**6.25** mg/ml
	CHLA	**6.25** mg/ml	**12.5** mg/ml	**6.25** mg/ml

The result of the minimum bactericidal (MBC) showed of the DES3: CHLA *M. oleifera* extract against *Staphylococcus aureus* (12.5mg/ml), *Pseudomonas aeriginosa* (12.5mg/ml) and *Escherichia coli* were (6.25mg/ml). And DES2: CHMA *P. odorata* extract against *Staphylococcus aureus* (6.25mg/ml), *Pseudomonas aeriginosa* (12.5mg/ml) and *Escherichia coli* were (6.25mg/ml). While DES2: CHMA *O.basilicum* extract against same bacterial strain showed (12.5mg/ml, 50mg/ml and 25mg/ml) respectively.

Table3: Minimum Bactericidal Concentration

Plants Scientific names	Solvent	*Staphylococcus aureus*	*Pseudomonas aeriginosa*	*Escherichia coli*
M. oleifera	LGH	**NA**	**25** mg/ml	**25** mg/ml
	CHMA	**25** mg/ml	**12.5** mg/ml	**12.5** mg/ml
	CHLA	**12.5** mg/ml	**12.5** mg/ml	**6.25** mg/ml
P. odorata	LGH	**25** mg/ml	**25** mg/ml	**12.5** mg/ml
	CHMA	**6.25** mg/ml	**12.5** mg/ml	**6.25** mg/ml
	CHLA	**25** mg/ml	**12.5** mg/ml	**12.5** mg/ml
O.basilicum	LGH	**50** mg/ml	**NA**	**25** mg/ml
	CHMA	**12.5** mg/ml	**50** mg/ml	**25** mg/ml
	CHLA	**25** mg/ml	**25** mg/ml	**12.5** mg/ml

3. Phytochemical screening

The results obtained for Total Phenolic Content and Total Flavonoid Content are offered in Figure 1. TPC of plants extracts by DES was different. The DES3: CHLA for *M. oleifera* extract, DES2: CHMA for *P. odorata* and *O.basilicum* (0.785±0.003, 0.989±0.007, and 0.701±0.008 mg GAE/g) showed the highest phenolic contents. Otherwise, the DES1: LGH for all plant kinds extract had the lowest TPC. In contrast, the speculation of TFC of plants extracts showed different in the range (0.013±0.004 and 0.064±0.009mg RE/g).

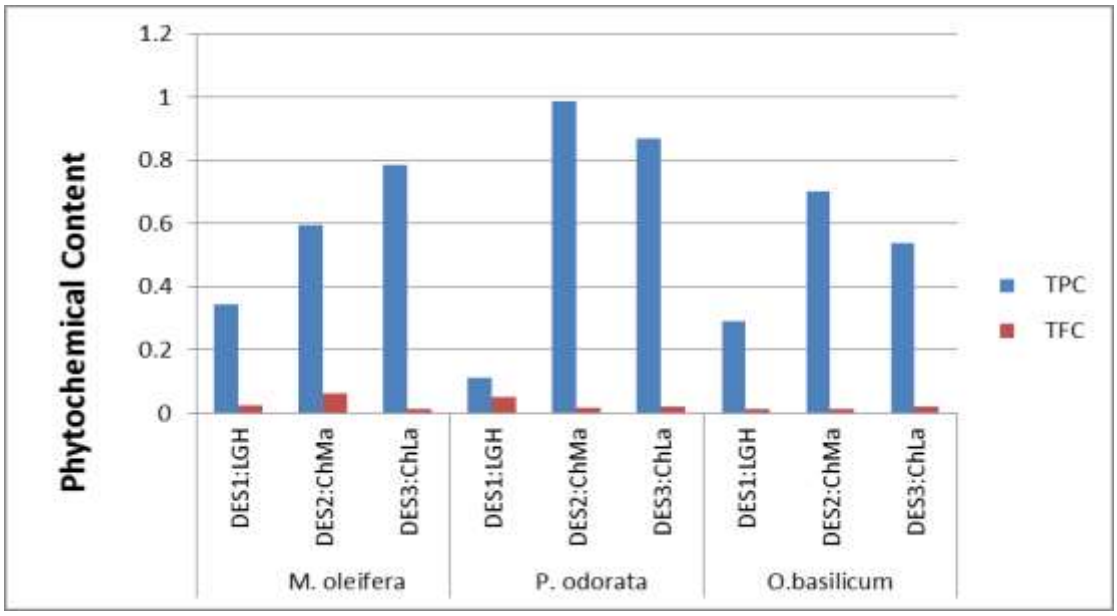

Figure1: Phytochemical content of all medicinal plants.

Discussion

The *M. oleifera* extract suggests has a range from phytochemical antibacterial effect and this result is recommended by previous studies, but he used traditional solvents (17,18). While DES3:CHLA that used in this study showed the best antimicrobial result against bacterial strain because the kind of DES is consider as a most efficient factor for various polarity bioactive compound extraction (19). *P. odorata* and O.basilicum plant extracts showed clear effect on Gram-positive bacterium *S. aureus* more than Gram-negative *S. typhimurium and E. coli* However, several previous findings (20,21). This is due to lipopolysaccharides bacterial material present their outer membrane (22). DES3 choline chloride based malic acid reported has super performance in extraction activity as well as support biological activities (23). The MIC, MBC results showed at various concentrations activity against three bacterial kinds presented at (table2 and3). The phytochemical analysis and quantitative estimation for all medicinal plants used in this study showed different outcome. Moreover, all related result showed good effective extraction for bioactive compound through use deep eutectic solvents compared to traditional solvents that have highly drawbacks (24).

Conclusion

Depended on the results gained in this work, all the medicinal plants extract by DES had different activity against bacterial strains used. These antimicrobial activities return to the composition solvents kind used for extraction, the in vitro results lead to the extracts can be used in alternative treatment. Based on the results of this study, it can be concluded *Moringa oleifera* Lam, *Premna odorata* Blanco and *Ocimum basilicum* L. extracted by DES2:CHMA and DES3:CHLA recorded big spectrum of activities in zone inhibition, MIC and MBC against different bacterial strains. These outcomes showed evidence about the secondary metabolites present in all medicinal plant used in this study through estimated TPC, TFC which may be have activities against bacterial strain.

Recommendations

The results of this project refer to should isolating bioactive compounds from crude extract and use for advanced experimental program. Therefore, we recommend for deep studies in isolation and fractionation of bioactive compounds. Moreover, in vitro cytotoxicity and in vivo studies.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

The present study does not involve in any experimental with animal or human organism.

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