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RESEARCH ARTICLE OPEN ACCESS

Phytochemical and Antimicrobial Activity Evaluation of The Water Immiscible Solvent Extracts of Moringa

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Abstract

Antimicrobial resistance (AMR) is the existing global apprehension for the social health. There is a requisite to take counteractive actions regarding the AMR. Based on the literature, it was aimed to perform the phytochemical and antimicrobial activity evaluation of the water immiscible solvent extracts of *Moringa peregrina* Forssk. Fiori (Family: Moringaceae). Seven extracts of the powdered leaves of *M. peregrina* were prepared in solvent systems comprising dichloromethane (DCM), dichloroethane (DCE), and their mixtures with ethyl acetate (EA) and chloroform (CH). The extracts were screened for their phytochemicals and antimicrobial potential. All the extracts showed positive tests for alkaloids, saponins, and flavonoids, wherein negative tests were obtained for tannins, cardenolides, and anthraquinone glycosides. The 1:1 mixture of EA:DCM, and EA:DCE provided positive tests for steroids and terpenoids. The most effective antimicrobial extracts were the 1:1 mixture of EA:DCM and EA:DCE. However, the antimicrobial activity of these extract was mild in comparison to ofloxacin and fluconazole. It is concluded that various mixtures of DCM, DCE, and CH along with the higher concentration of other miscible solvents of DCM, DCE, and CH may provide better antimicrobial extracts.

Keywords: *Moringa peregrina*, Leaves, Water immiscible solvent, Extract, Antibacterial, Antifungal.

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INTRODUCTION

Antimicrobial resistance (AMR), an existing global worry for social health, is frequently linked to the irrational practice of antibiotics[1,2]. The reports of AMR bacterial infections are accumulating in the Kingdom of Saudi Arabia owing to the irrational practice of antibiotics in addition to the societal, economic, and demographic features of Saudi, non-Saudi and visitor population[3-6]. Additional factor causative to the expansion of AMR is the failure to find out innovative antimicrobial means[7-9]. Consequently, there is a necessity to take counteractive actions regarding the issues associated to AMR.

The indiscriminate use of synthetic antibiotics poses a serious threat to humans as multidrug resistance has developed among the disease-causing microbes[10] towards synthetic antibiotics. Therefore, scientists are more focused on plant-based drugs which are not or least toxic to treat infectious diseases. *Moringa peregrina* Forssk. Fiori (Family: Moringaceae) is reported to possess a wide variety of biological activities that include antioxidant property, antimicrobial property, antidiabetic property, antispasmodic property, hepatoprotective property, antihypertensive property, lipid-lowering activity, anti-inflammatory activity, treatment of the mental disorders, and anticancer activity[11-14]. The complete plant description, its cultivation, the economic status, the different phytoconstituents, and the biological activity profile of the different solvent extracts of *Moringa peregrina* has also been disclosed in the published review articles[14,15]. According to one citation of 2018, most of the antimicrobial activity reports of the leaves of *M. peregrina* are related to the water-miscible solvents extracts and the compounds isolated from these extracts[14]. However, the literature of *Moringa peregrina* is silent about the phytochemical and antimicrobial activity evaluation of the water immiscible solvent extracts of the leaves of *M. peregrina*[11-15]. In view of the above facts, it has been decided to perform the phytochemical and antimicrobial activity evaluation of the water immiscible solvent extracts of the leaves of *M. peregrina* that can lead to the identification of the possible new class of the phytoconstituents as antimicrobial agents.

MATERIALS AND METHODS

Collection of the Plant Material

The semi-dried leaves (2 kg) of *M. peregrina* were obtained from Al Oula region (Saudi Arabia) in January 2019. These leaves were authenticated by Prof. Abdulhakim Bawadekji and specimens were kept in the herbarium with n. Oul 1. The semi-dried leaves were cleaned and air dried for 8 days at 25-30°C. The dried up leaves were broken up into a coarse powder with the help of a grinder. The powder was sieved and stored in polyethene bags for the extraction purpose.

Preparation of the Extracts

The powder of the leaves (50 g) was transferred to a 1000 ml flask. dichloromethane (DCM, 500 ml) was added to the flask. The flask was plugged and the content was stirred at 25-30°C for 30 minutes. The obtained mixture was kept 25-30°C for three days with occasional shaking. The mixture was filtered with Whatman filter paper. The resulting filtrate was concentrated in a rotary evaporator and a semisolid residue was obtained.

The extracts of dichloroethane (DCE), and the 1:1 mixtures of chloroform: dichloromethane (CH:DCM), chloroform: dichloroethane (CH:DCE), dichloroethane: dichloromethane (DCE:DCM), ethyl acetate: dichloromethane (EA:DCM), and ethyl acetate: dichloroethane (EA:DCE) were also obtained in a similar manner.

Phytochemical Studies

The extracts of DCM, DCE, CH:DCM (1:1), CH:DCE (1:1), DCE:DCM (1:1), EA:DCM (1:1), and EA:DEC (1:1) were screened for their phytoconstituents, including alkaloids (Mayer's test & Wagner's test), tannins ($FeCl_3$ test and Lead acetate test), cardenolides (Baljet test and KellarKillani test), steroids (Liebermann-Burchard test), terpenoids (Salkowski's test), saponins (Foam test), Anthraquinone (Borntrager's test), and Flavonoids (Flavonoid test). The screening was carried by the standard procedures[15,16].

Antimicrobial Screening

The antimicrobial activity was performed by the serial plate dilution technique[17,20]. Six microorganisms were used to determine the minimum inhibitory concentrations (MIC) of the extracts and the standard drugs, ofloxacin

and fluconazole. Different concentrations of the extracts and the standard, ranging from 10-200 μgml^{-1}, were prepared using sterile dimethylformamide (DMF) as a solvent, which also served as a control group. Agar media and Sabouraod dextrose media were used for antibacterial and antifungal activity assessment, correspondingly. The tested microorganisms were the followings: *Escherichia coli* Castellani and Chalmers ex. Migula, *Klebsiella pneumoniae* Trevisan ex. Shroeter, *Pseudomonas aeruginosa* Migula ex. SCHRUTER, *Staphylococcus aureus* Rosenbach, *Aspergillus niger* Tieghem, *Candida albicans* (Robin) Berkhout.

Statistical Analysis

The data (N = 3, Mean±Standard Error Mean) was analysed by SPSS-software, in which $p < 0.05$ specified the significant results.

RESULTS AND DISCUSSION

Many reports about the antimicrobial potential of the leaves of *M. peregrina* have been documented. However, the literature is silent about the phytochemical and antimicrobial activity evaluation of the water immiscible solvent extracts of the leaves of *M. peregrina.* Accordingly, seven extracts of the powdered leaves of *M. peregrina* were prepared using dichloromethane, dichloroethane, and their mixtures with ethyl acetate and chloroform. The physical data of these extracts is provided in Table 1. According to this data, all the semisolid extracts had a greenish appearance, and the percentage yield range from 8% to 14%. The highest yield (14%) was obtained for the 1:1 mixture of ethyl acetate and dichloroethane (EA:DCE), wherein the lowest yield (8%) was obtained for the 1:1 mixture of chloroform and dichloromethane (CH:DCM). This variation in the yield might be because of the polarity of the solvents systems used for the extraction.

The phytochemical analysis of the extracts was performed by the standard procedure[15,16], which is mentioned in Table 2. According to this data, all the extracts showed positive tests for alkaloids, saponins, and flavonoids. This shows that alkaloids, saponins, and flavonoids are among the main chemical constituents of the

Table 1. Physical data of the extracts of *M. peregrina*

Extract	Percentage Yield	State	Colour
DCM	10	Semisolid	Light Green
DCE	10	Semisolid	Light Green
DCM:DCE (1:1)	10	Semisolid	Light Green
CH:DCM (1:1)	8	Semisolid	Dark Green
CH:DCE (1:1)	10	Semisolid	Dark Green
EA:DCM (1:1)	12	Semisolid	Greenish brown
EA:DCE (1:1)	14	Semisolid	Greenish brown

Table 2. Phytochemical screening data of the extracts of *M. peregrina*

Phytochemical	DCM	DCE	DCM:DCE (1:1)	CH:DCM (1:1)	CH:DCE (1:1)	EA:DCM (1:1)	EA:DEC (1:1)
Alkaloids	+	+	+	+	+	+	+
Tannins	-	-	-	-	-	-	-
Cardenolides	-	-	-	-	-	-	-
Steroids	-	-	-	-	-	+	+
Terpenoids	-	-	-	-	-	+	+
Saponins	+	+	+	+	+	+	+
Anthraquinone	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+

(+) = Present & (-) = Absent

leaves of *M. peregrina*[14]. All the extracts showed negative tests for tannins, cardenolides, and anthraquinone glycosides. The 1:1 mixture of ethyl acetate with dichloromethane (EA:DCM), and dichloroethane (EA:DCE) gave positive tests for the presence of steroids and terpenoids. All other extracts exhibited a negative test for the steroids and terpenoids. However, the presence of tannins, steroids, and terpenoids along with other chemical constituents has been mentioned in water-miscible solvents[15-21].

The antimicrobial activity of the extracts was performed by serial dilution technique[17,18] against four bacteria and two fungi using ofloxacin and fluconazole as standard drugs, respectively. The MIC values of the extracts, ofloxacin, and fluconazole with respect to the tested microorganisms are mentioned in Table 3. For the comparison purpose, the MIC values of ofloxacin and fluconazole are taken as 100%.

Table 3. Antimicrobial screening data of the extracts of *M. peregrina*

Extract / Standard	MIC (µgml^{-1})(% MIC with respect to the standard)					
	Escherichia coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Aspergillus niger*	*Candida albicans*
DCM	125(16%)	150(13.33%)	150(13.33%)	150(13.33%)	125(12%)	125(12%)
DCE	100(20%)	125(16%)	100(20%)	100(20%)	100(15%)	100(15%)
DCM:DCE (1:1)	125(16%)	125(16%)	125(16%)	125(16%)	100(15%)	100(15%)
CH:DCM (1:1)	150(13.33%)	150(13.33%)	150(13.33%)	150(13.33%)	150(10%)	125(12%)
CH:DCE (1:1)	125(16%)	125(16%)	125(16%)	100(20%)	125(12%)	125(12%)
EA:DCM (1:1)	100(20%)	100(20%)	75(26.66%)	75(26.66%)	75(20%)	75(20%)
EA:DEC (1:1)	75(26.66%)	100(20%)	75(26.66%)	75(26.66%)	75(20%)	75(20%)
Fluconazole	-	-	-	-	15(100%)	15(100%)
Ofloxacin	20(100%)	20(100%)	20(100%)	20(100%)	-	-

*p<0.05 & N = 3.

All the data had a statistically significant ($p < 0.5$) results. It is evident from Table 3 data that all the seven extracts possess mild antifungal activity in comparison to fluconazole (MIC: 15µgml^{-1}; 100%) against *C. albicans* and *A.niger.* The most effective antifungal extracts were the 1:1 mixture of ethyl acetate:dichloromethane (EA:DCM) and ethyl acetate:dichloroethane (EA:DCE), which had MIC of 75µgml^{-1}. However, it was only 20% in comparison to fluconazole against *C. albicans* and *A.niger*. It is also evident that all the seven extracts possess mild antibacterial activity in comparison to ofloxacin (MIC: 20 µgml^{-1}; 100%) against *S. aureus, E. coli, P. aeruginosa,* and *K. pneumoniae.* The most effective antifungal extracts were the 1:1 mixture of ethyl acetate: dichloromethane (EA:DCM) and ethyl acetate: dichloroethane (EA:DCE), which had MIC of 75-100 µgml^{-1}. However, it was 20-26.66% in comparison to ofloxacin against *S. aureus, E. coli, P. aeruginosa,* and *K. pneumoniae*. It was also observed that solvent system, for example, EA:DCE system, having more polarity had better antifungal and antimicrobial activity in comparison to the less polar solvent system, for example, DCM. The higher antimicrobial activity of the EA:DCM and EA:DCE solvent system might be because of the presence of steroids and terpenoids, which are absent in other solvent systems[14] (Table 2). Another possibility of this result is that the extracts of the more polar solvent system might be having more number chemical constituents, which attribute to the higher antimicrobial activity of the extracts of the more polar solvent systems[11].

CONCLUSION

It is evident from the results that the extracts of 1:1 mixture of EA:DCM and EA:DCE possess more phytoconstituents in comparison to other extracts of DCM, DCE, DCM:DCE (1:1), CH:DCM (1:1), and CH:DCE (1:1). Accordingly, it is apparent that the better antimicrobial potential of the extracts of 1:1 mixture of EA:DCM and EA:DCE is because of the presence of more number of phytoconstituents. It is concluded

that various mixtures of DCM, DCE, and CH along with the higher concentration of other miscible solvents of DCM, DCE, and CH may provide better antimicrobial extracts.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors have made substantial, direct and intellectual contribution to the work and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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