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Phytochemical Screening Of Extracts Of *Malva neglecta* And Evaluation Of Their Biological Activity.

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ABSTRACT

The present study was designed with the aim to evaluate the phenolic content (TPC), identify some of the phenolic acids by high performance liquid chromatography (HPLC) and to evaluate the antimicrobial activity of the aqueous, methanolic and chloroform extracts of *Malva neglecta*. The total phenolic compounds were measured by Folin ciocalteu method and some of them were identified by HPLC. All the three extracts were evaluated for their antimicrobial activity by the cup plate method against *Staphylococcus aureus, Bacillus subtilis, Eschericia coli* and *Proteus vulgaris* organisms. The maximum amount of TPC was found in the aqueous extract i.e. 580 µgm followed by methanolic and chloroform extracts i.e.448 µgm and 180 µgm respectively. Results of the HPLC analysis of the methanolic extract revealed the presence of phenolic acids i.e. Gallic, ellagic, tannic and salicylic acids. The methanolic and aqueous extracts were more effective against *E. coli , P. vulgaris* and *S. aureus*, and chloroform extract was effective against only E. coli. From the results it can be assumed that the phenolic acids present in these extracts may be one of the contributing factors for the antimicrobial activity.

Keywords: *Malvaneglecta*, HPLC, Phenolic compounds, anti-microbial

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INTRODUCTION

Plants play an important role in the health services around the globe as the source of medicine. The knowledge of the drugs has been accumulated over thousands of years as a result of man's inquisitive nature. Furthermore from time immemorial the rural population depends largely on herbal remedies for the treatment of various diseases and disorders which indicate the importance of the individual plants in the health care system [1, 2]. New drugs from natural sources are even today considered important despite the advances in modern medicine. Hence studies regarding the bioactivities of plants have assumed an important place in plant research. Plants are considered as a rich source of medicines as they synthesize molecules that have biological activity. The interest and concern to discover novel compounds from natural origin is ever on the increase [3]. Therefore making the field wide open for research. Phenolic compounds are an important group of secondary metabolites. They contain benzoic nucleus with one or several hydroxyl groups and their derivatives. In the recent past there has been an increase in the interest towards these compounds as their dietary intake is related to decreased incidence of chronic degenerative diseases[4].Phenolic compounds are vital in defense responses, such as anti-aging, anti-inflammatory etc [5]. The phenolic compounds exhibit various pharmacological activities some of them being anti-inflammatory, antioxidant, anti-proliferative activities, anti-cancer and Anti atherogenic [6-8].

Malvaneglecta (Arabic name: Khebaiz, khabizat muhmala) is a plant belonging to the Malvaceae family and is wildly grown in the Northern Border Province region of Saudi Arabia [9, 10].Literature reports the anti-ulcerogenic, antibacterial, antioxidant properties of this plant [11-13].The macro, micro mineral content (i.e. Na, K, Cu, P, Fe etc), 4-OH benzoic acids, essential oil and fatty acids have also been reported [14-16]. Traditionally it has been used for insect bites, bladder infection, burns, cold, cough inflammation, wounds and as astringent, demulcent, diuretic, expectorant, laxative and in inflammations [17, 18].

The present study was designed with the aim to quantify the TPC, identify some of the phenolic acids by HPLC analysis and also to evaluate the antimicrobial activity of the extracts.

MATERIALS AND METHODS

1. Collection of plant: *Malva neglecta* which is wildly grown was collected from Rafha, Northern Border Province, Saudi Arabia. The plant material was shade dried, pulverized and stored in the research laboratory of College of Pharmacy, Rafha.

2. Extraction of the plant material: The dried plant material was successively extracted using these solvents i.e. Petroleum ether, Chloroform, ethyl acetate, methanol and water.

3. Phytochemical analysis

3(a) Preliminary phyto chemical analysis: The various extracts of the plant were subjected to qualitative analysis for the phytoconstituents like alkaloids, carbohydrates, glycosides, steroids, tannins, proteins, amino acids and flavonoids as per the standard procedures.

3(b) Determination of total phenolic content in methanolic extract [19]

Folin ciocalteu method was used for the evaluation of total flavonoids. Gallic acid was used as a standard and the absorbance was measured at 750 nm in the UV spectrometer by plotting absorbance versus the concentration. The total phenolic content was expressed as gallic acid equivalent

3(c) HPLC Analysis: Shimadzu High Performance Liquid Chromatographic system equipped with LC-10ATVP pump,7725i rheodyne injector, Shimadzu spd10 A uv-vis detector in combination with data system N2000 software was used for the analysis. Running conditions included: injection volume 50 µl(Hamilton), mobile phase, methanol: Phosphate buffer(0.02M) pH3, flow rate, 1 ml/min, detection at 254nm, Elution Type: Isocratic. Gallic,ellagic acid, tannic and salicylic acid were used as standards. The Mobile phase was prepared by mixing 0.2 M Potassium phosphate buffer (pH 3): Methanol (60:40).

Antimicrobial study of plant extract by cup plate method

The antimicrobial activity of the plant extract was evaluated by cup plate method using both gram positive (i.e.*Staphylococcus aureus* and *Bacillus subtilis)* and gram negative *(Eschericia coliand Proteus vulgaris*) organisms [20]

The required quantity of Nutrient agar media was prepared and it was sterilized along with petri dishes and borer in an autoclave for 15 minutes at 121^0C .The extracts were initially dissolved in H_2O / DMSO and tested at concentration of 200 and 100 µg/ml against all the microorganisms.

The prepared sterile plates were inoculated with 0.1 ml of the inoculum from standard culture of test organism. A sterile borer of diameter 10mm was used for preparing the wells and 100µl of the test substance, standard antibiotic and the solvent control were added in each well separately. The plates were placed at 4^0C for 1 h so as to allow the diffusion of test solution into the medium this was followed by the incubation of plates at a temperature optimal for the test organism and for a period of 24 hours at 37°C. The diameter of the zone of inhibition of microbial growth around the well was measured in mm.

RESULTS AND DISCUSSION

The plant material was collected dried and subjected to exhaustive extraction which is one of the common methods of extraction. This method involves successive extraction with solvents of increasing polarity from a non-polar to a more polar solvent so as to ensure that a wide polarity range of compounds could be extracted [21].The solvents used were petroleum ether, chloroform, ethyl acetate, methanol and water. The crude extracts were dried and the percentage yield was calculated and is as represented in table no 1. The maximum % yield was obtained from the methanolic extract (20.4%), followed by aqueous (12.9%), petroleum ether (6.70%), chloroform (4.80%),ethyl acetate (2.7%).This indicates that methanol is a suitable solvent for extraction of phytoconstituents from this plant.

Table 1: Nature and percentage yield of the extracts

Sl No	Solvent	Nature	%Yield
1	Petroleum ether	Greenish oily mass	6.70
2	Chloroform	Chocolate brown sticky mass	4.80
3	Ethyl acetate	Brownish black mass	2.7
4	Methanolic	Light brown mass	20.4
5	Aqueous	Blackish brown mass	12.9

The plant extracts were then subjected to preliminary phytochemical analysis which revealed the presence of glycosides, tannins, flavonoids, saponins, sterols, carbohydrates and proteins in the methanolic and aqueous extracts. Ethyl acetate extract gave positive tests for the presence of tannins, sterols, and phenolic compounds, the petroleum extract contained fixed oil and fats, while the chloroform extract contained gave positive results for phenolic compounds.

Considering the importance of phenolic compounds which have been reported to possess several of the activities like analgesic, anti-inflammatory, anti-oxidant and anti-microbial activities (22), the total phenolic content was estimated for the chloroform, methanolic, aqueous extracts(as phenolic compounds were detected in these extracts during the preliminary phytochemical analysis) by Folin Ciocaltteu method. The absorbance of the chloroform extract was 0.0628 and the amount of TPC was 180µgm, absorbance of methanolic extract was seen at 0.1486and the amount of phenolic acid was found to be 448 µgm .The maximum amount of TPC was found in the aqueous extract i.e. 580µgm which showed an absorbance at 0.1828.The phenolic content of the extract increased with the polarity of the solvent; the highest amount of TPC corresponded to water. Our result is in agreement with the reports of other researchers who showed that polar solvents are suitable solvents for extraction of phenolic compounds [23, 24]. Fig 1 represents the amount of phenolic acids in the different extracts.

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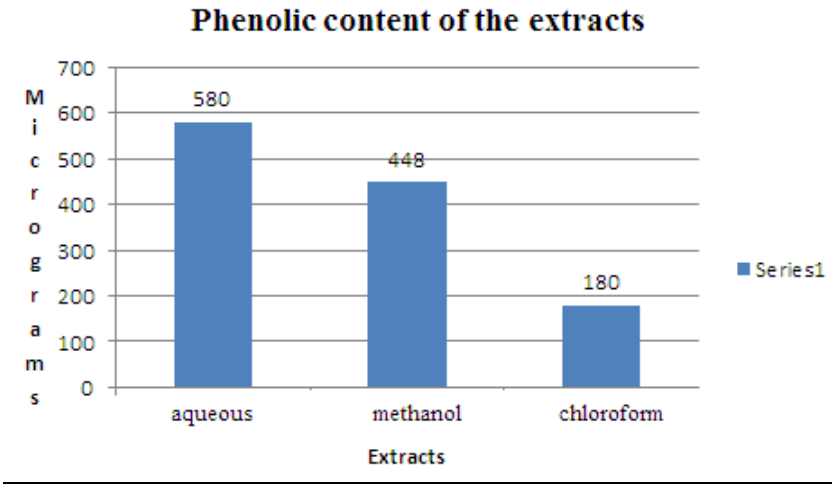

Fig 1: Phenolic content of the extracts

HPLC is one of the important and popular methods for analysis of plant extract. Each phytoconstituents has a characteristic peak and is detected by the detector at a particular retention time. The HPLC analysis was carried at conditions as described in the experimental section using phenolic acids like gallic acid, ellagic acid, tannic acid and salicylic acid as the standard biomarkers representing this class of phytoconstituents (phenolic acids).Figure 2 shows the HPLC chromatogram of the methanol extract at wavelength detection of 254.The phenolic compounds gallic acid, ellagic acid; tannic acid and salicylic acid were identified in the methanolic extracts of the plant. Gallic acid showed a retention peak at 3.123, while ellagic acid, tannic acid and salicylic acid showed retention peaks at 5.08, 9.82 and 13.198 respectively. The retention time, height and area under the curve for the various phenolic acids is as shown in the table 2.

Fig 2: HPLC chromatogram for the methanolic extract

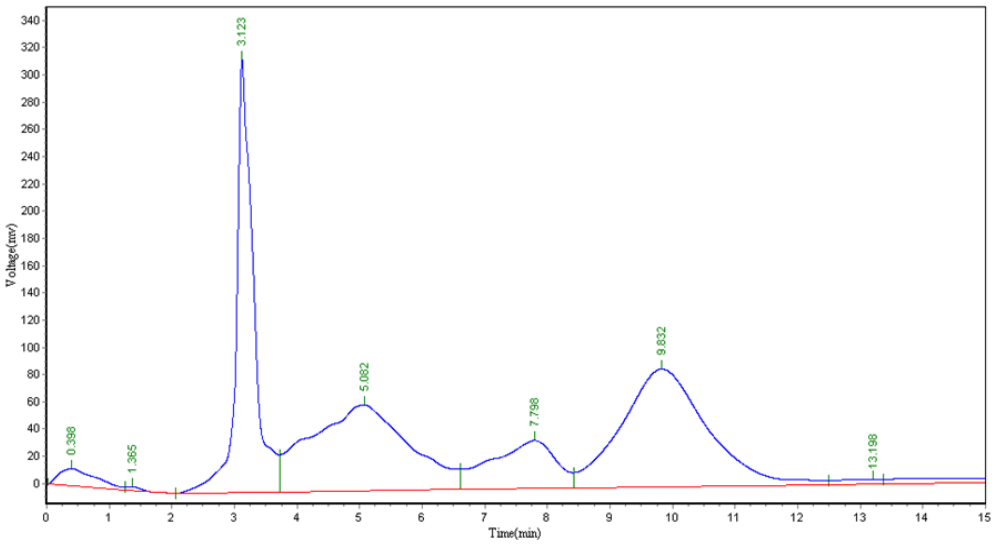

Table 2: Retention time, height and area under the curve for the various phenolic acids:

Peak id	Retention time	Height	Area	Concen
Gallic acid	3.1230	317833.000	6070458.50	24.0526
Ellagic acid	5.0820	63024.176	6859445.00	27.1788
Tannic acid	9.8320	86520.352	8258778.50	32.7233
Salicylic acid	13.198	3601.372	180356.016	0.7146

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Table 3: Anti-microbial effect of the extracts by cup plate method

	Zone of inhibition in mm							
Extract	*E. coli*		*P. vulgaris*		*S. aureus*		*B.cereus*	
	100	200	100	200	100	200	100	200
Chloroform	NI	7	NI	NI	NI	NI	NI	NI
Methanol	09	14	NI	10	07	12	NI	NI
Aqueous	08	12	NI	08	04	09	NI	NI
Streptomycin	13	24	NI	16	15	19	4.5	07

NI:No inhibition

The antimicrobial activity of the various solvent extracts was performed by the cup plate method. Bacterial organisms sometimes grow on some crude methanolic extracts which can be attributed to the natural resistance of the organism / absence of antimicrobial constituents in the experimental extract. Results of the present study are as reported in Table 4 and has shown variations in antimicrobial activity of the various extracts. The methanolic extracts were more effective against E. coli, P. vulgaris and S. aureus at 200 mcg/ml followed by the aqueous extract, while the chloroform extracts was active against only one organism i.e. E. coli at 200 mcg/ml. None of the extracts showed antibacterial activity against B.cereus.The Gram-positive bacteria are usually more susceptible as they posses only an outer peptidoglycone layer which may not be an effective permeability barrier. It has been reported that certain Gram-negative bacteria are not susceptible to plant extracts and this may be related to in their outer membrane of the cell [25-28]. The inactivity of the extracts against B.cereus could be attributed to this difference in the cell membrane of the bacteria used for the antimicrobial investigation. It has been also been reported that efflux pumps present on the bacterial cell membrane may be another contributing factor for resistance in bacteria. Bacteria make use of these efflux pumps to remove the antibiotic component from the cell until the concentration becomes such that it is no longer effective against the bacteria. The whole extracts contain a number of phytoconstituents which work in combination at small concentrations having lack of specificity as compared to the purified compounds. Sometimes compounds have the capacity of disrupting the integrity of the bacterial membrane/ increase bacterial membrane permeability / cause rapid depolarisation of the bacterial membrane and eventual cell death [29].

Hence the results of the current study suggested that the efficacy of any plant extract depends on the type of solvent used for extraction and the tested microorganism. It was found that methanol was more efficient when compared to water and chloroform in extracting antimicrobial phytochemicals from this plant. The chemical structure of the antimicrobial agents found in plants belongs to most commonly secondary metabolites such as flavonoids and phenolic acids. Plant extracts are great sources of phenolic acids and represent the highest antibacterial effects against Gram-positive bacteria. The methanolic extract revealed the presences of flavonoids and phenolic acid; the cell disrupting activity may be attributed to the presence of these constitiuents [30].It has been reported that antimicrobial components of the plant extracts like phenolics, interact with enzymes and proteins which are present in the microbial cell membrane causing its destruction, or may cause inhibition of enzymes necessary for biosynthesis of amino acids. Other groups of researchers attributed this inhibitory effect to hydrophobic properties of the extract which is thought to lead to interference in the structures thereby changing their permeability [31-35].

CONCLUSION

The results of the current study revealed that the aqueous, methanol and chloroform extracts of the *Malva neglecta*, exhibited antibacterial activity against the tested microorganisms. HPLC analysis confirmed the presence of Gallic, ellagic, tannic and salicylic acids in the methanolic extract. Based on our results, we can assume that the specific antimicrobial activity is likely to depend on the presence of these phenolic acids and other constituents of these extracts. This work high lights the role of bioactive components like phenolic acids as source of drug for antimicrobial constituents. Screening of the traditionally used plants for their medicinal properties may prove to be a pathway for scientific validation for the use of these plants.

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