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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND THIN LAYER CHROMATOGRAPHIC OF *PRUNUS DULCIS* (ALMOND) MEDICINAL PLANT LEAVES USED IN FOLK MEDICINE FOR TREATMENT OF WOUNDS AND BURNS IN HUFASH DISTRICT AL MAHWEET GOVERNORATE–YEMEN

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ABSTRACT

In this study methanolic and aqueous extracts of one plant namely *Prunus dulcis* were screened for the presence of phytochemical constituents and tested for their antimicrobial and antioxidant activity. The qualitative phytochemical analysis revealed the results showed presence of alkaloids, terpenoids, glycosides, resins, saponins, tannins, flavonoids, phenols, and amino acid were present in the methanol extract, with absence of glycosides, and amino acids in the aqueous extracts in leaves plant. TLC tests conducted revealed Rf values in the leaves for alkaloids, flavonoids, tannins, phenols and saponins(0.92-0.96-0.96-0.95-0.96) respectively. The antimicrobial activity extracts against four bacterial isolates *Staphylococcus aureus*, *Escherichia coli* , *Pseudomonas aeruginosa* and Klebsiella sp. and a single fungal isolate *Candida albicans* with concentrations (0.5 mg/ml, and 1,0 mg/ml) of the extract were added to the disc and respective solvent was used as negative control. The antioxidative activity of leaf was evaluated by using 1,1- diphenyl-2 picrylhydrazyl (DPPH), the results showed are 85.5%, lowest from standard, ascorbic acid 87.5%.

Keywords: Antimicrobial, antioxidative, phytochemical, *Prunus dulcis.*

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INTRODUCTION

Prunus dulcis (Almond) belongs to the family Rosaceae, which is a widely grown fruit tree that is commercially important throughout the world. It is native to mountainous regions of Central Asia[1]. The skin of the *Prunus dulcis* nut accounts for 4% of the total nut weight and is rich in polyphenols, including hydroxybenzoic acids and aldehydes, flavonol and flavanone aglycones, and glycosides[2].

Antioxidant activity of almond extract was investigated by DPPḢ, ABTṠ+, OḢ radical scavenging, metal chelating activity and determination of lipid peroxidation levels (TBARS).

Almond extract scavenged 89.50% of the ABTS radical, 66.77% of the hydroxyl radical, and 87.30% of the DPPH radical[3].

MATERIALS AND METHODS

Samples extraction

The Samples of 100g of the grinded powder were put in sterilized flasks together with 400 ml of pure methanol for methanolic extraction treatments, while for aqueous extraction treatments, samples of 100g of grinded powder were put in sterilized flasks with 400 ml of distilled water each. All flasks were covered with transparent nylon and tin and then all were put on a rotary shaker machine for 24 hours, the speed of the device was 200 r/m at the laboratory temperature (22.7°C). The filtration process for each sample was carried out using filter paper to obtain a pure solution. The evaporation process for each methanol solution and distilled water was conducted separately in the evaporator (methanol solution at 42°C and pressure 337. The distilled water solution at 45°C and pressure

72 for 2 hours for methanol solution and 4 hours for distilled water solution. Then obtained extracts were kept in dark conditions in the refrigerator at 4°C until used in the experiment[4].

QUALITATIVE TESTS

Phytochemical screening of plant extracts:

The methanolic and aqueous extracts subjected to phytochemical screening were alkaloids, terpenoids, glycosides, resins, saponins, tannins, flavonoids, phenols, and amino acids[5,6].

Thin Layer Chromatographic.

One gram of *Prunus dulcis* powder was boiled with of with solvent system made from 15ml H_2SO_4 test for Alkaloids, 10ml 70% ethanol test for flavonoids and saponins, 25 ml water test for Tannins and Phenols15ml H_2SO_4 test for Alkaloids in rounded flasks. The TLC plate was prepared as such : (Layer : silica gel layers 0.25 mm thickness, 10 cm length and 5cm wide). The filtrate obtained was evaporated to dryness in a water bath at 37° C .The residue was dissolved by 0.2ml methanol. The solution was used for spotting the TLC by capillary tube by only one centered spot. The TLC plate was put inside a saturated tank, and development was waited. When the mobile phase reaches two thirds of plate's length, the plate was lifted out from the tank and let to dry in air. The plate was examined by U.V. lamp at the wavelength 365nm. The colors of florescence appeared and recorded. The plate was sprayed carefully reagent, and let to dry for 10 min, then sprayed with solution. After it plate was examined under U.V. lamp at the wave length 365nm. The iodine was used as the visualizing agent to detect the spot. A meter rule was used to measure the distance moved by the solvent and distance moved by spot, from which the retention factor (R_f values) of the various spots was calculated[7]. TLC was performed for alkaloids, flavonoids, tannins and phenols solvent system and confirmatory tests are shown in Table 2. Calculation of RF of each spot was as follows:

$$Rf = \frac{\text{Distance moved by solute from the origin}}{\text{Distance moved by solvent from the origin}}$$

Antimicrobial Activity of Plants extracts

Microbial Cultures: Fresh plates of the four bacterial isolates *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella sp.* and a single fungal isolate *Candida albicans* were obtained from the National Center of Public Health Laboratories, Sana'a.

Media Use: The bacterial test were *spread* over the nutrient ager (56g/1000ML distilled Water) was weight into separate flask and dispensed into distilled water make a total volume of 1 liter. Then the fungal test were spread over the sabouraud dextrose agar (65g/1000ML distilled Water) was weighted into separate flask and dispensed into distilled water to make a total volume of 1 liter. These powders were dissolved in distilled water and used for evaluation of their antibacterial and antifungal activities. The mixture was heated in an electric water bath (GFC, 1083, Germany) until the Agar melted to form a homogenous solution. The prepared medium was separately transferred to Durum medium bottle and sterilized by autoclaving at 121^0 C for 30 minutes. The sterile medium was allowed to cool to about 45^oC before being poured aseptically in an inoculation. Chamber (Ceslab England) in 15 ml portions, into sterile petri dishes to cool and gel into solids[8].

Antimicrobial activity assay: Two different concentrations (0.5 mg/ml, and 1,0 mg/ml) of the extract were added to the disc and respective solvent was used as negative control.

Zone of Inhibition: The bacteria plates were incubated at 37^oC for 24hrs while the fungal plates were incubated at for 72 hours, and observed for the zone of inhibition of growth, The zones were measured with a transparent ruler and the result recorded.

Determination of antioxidant activity

The scavenging ability of the natural antioxidants of the leaves towards the stable free radical DPPH was measured by the method as used in a previous study[9]. The leaf extracts (20µl) were added to 0.5ml of methanolic solution of DPPH (0.3mM in methanol) and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 min. Methanol served as the blank and DPPH in methanol, without the leaf extracts, Served as the positive control. After 30 min of incubation, the discolouration of the purple colour was measured at 517 nm in a spectrophotometer**)**. The radical scavenging activity was calculated as follows:

Radical Scavenging Activity (RSA100%) =

$$\frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Statistical Analysis

Analysis of variance was made for all data using (SPSS) version (25) computer program.

RESULTS AND DISCUSSION

In this study methanolic and aqueous extracts of one plants namely *Prunus dulcis*, were screened for the presence of phytochemical constituents and tested for their microbial and antioxidant activity.

Yield from different solvents

Yield of methanolic extract of *Prunus dulci*, extracted with 100% methanol produced 28.65 (g). While yield of distilled water extract of *Prunus dulcis* produced 25.33(g).

Mean values of the yield are presented as mean ± SEM. Values are statistically significant when $p \leq 0.05$.

Unfortunately no literature was found on yield of neither methanolic nor aqueous extracts of leaves of Prunus *dulcis.*

Phytochemical composition of the methanolic and aqueous leaves extracts.

The summarized phytochemical screening of chemical constituents of *Prunus dulcis* extract is shown in Table 4. The results revealed the presence of active compounds in the two different extracts. As the table shows, the methanol and aqueous extracts indicate the presence alkaloids, terpenoids, glycosides, resins, saponins, tannins, flavonoids, phenols, and amino acid were present in the methanol extract, with absence of glycosides, and amino acids in the aqueous extracts in all three plants .

In a study done in a previous study in their study to detect chemical constituents of the leaves of *P. dulcis*

by the approach based on liquid chromatography-mass spectrometry (LC–MS) combined with isolation and structure elucidation of pure compounds by Nuclear Magnetic Resonance (NMR) analysis detected phenolics, terpenoids and a cyanogenic glycoside which is more specific than our findings[10]. The almond (*P.dulcis)* extract studied by indicated the presence of phytochemicals including phenolic compounds and flavonoids[11].

Thin layer chromatography (TLC)

Five secondary metabolites (alkaloids, flavonoids, tannins, phenols and saponins) were used for (TLC) thin layer chromatographic analysis. TLC tests conducted revealed *Rf* values in the leaves of *Prunus dulcis* for alkaloids, flavonoids, tannins, phenols and saponins (0.92-0.96-0.96-0.95-0.96) respectively.

Prunus dulcis leaves have been reported to exert some biological activity, in particular potent free radical-scavenging capacity, but so far there is limited information on their chemical composition**.**

Antibacterial and antifungal activity of plants extracts.

Antimicrobial activity of standard antibiotics discs against tested bacterial and Fungal are displayed in Table 5.The results of the study indicated that control Antibiotics against bacteria and Fungi showed different inhibitory zones. Antibiotics activity of AM (10ug) , CIP(25ug), CF(30ug), PZ (75ug) and PC (100ug) against *Staphylococcus aureus* were 19, 26, 20, 21, 20 mm ; *E. coli* 17, 28, 18, 20, 19 mm; *Pseudomonas aeruginosa* 18, 30, 17, 21, 18 mm; *Klebsilla sp*. 20, 33, 22, 23, 17 mm, and *Candida albicans* 21, 31, 20, 19, 22 mm respectively.

It is clear from Table 6, that the antimicrobial activity of the two methanol concentrations of *Prunus dulcis* gave lower inhibition zones than all antibiotics used in the study.

As shown in Table 7**,** the antimicrobial activity of both aqueous concentrations of *Prunus dulcis* were lower in inhibition zones than all antibiotics except *E. coli* which had the closest activity to AM *Staphylococcus aureus*..a nearly to AM and *candida albicans* nearly to PZ. This study showed that Ciprofloxacin (30µg) gave the highest inhibition zone among all antibiotics with the selected organisms 26, 28, 30 mm against *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa respectively*. In a similar study[12] Ciprofloxacin (25µg) gave high diameter of inhibition zone which reached up 19, 23, 23 mm against *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa* respectively. The majority of the antibacterial activity in this study was found in the methanolic rather than the aqueous extracts, and the highest activity was found in the methanolic extracts from *Prunus dulcis.* Similar results were achieved by in another study[13]. In the present study it was observed that the extract of *Prunus dulcis leaves* showed antimicrobial activities, with varies values, against all the tested organisms, as indicated in Table 7. Unfortunately no literature was found on extracts of neither methanolic nor aqueous extracts of leaves of *Prunus dulcis* in antimicrobial activities.

Antioxidant activity

Results showed are 85.5%, lowest from standard, ascorbic acid 87.5% **(**Table 8).

In a previous study done it was found that the total antioxidant activity of *prunus amygdalus* leaves extract was 1377 mg/ml while 85.5% of *prunus dulcis* in present study[14].

CONCLUSION

The present study showed that *prunus dulcis* are rich sources of useful secondary metabolites, it is strongly recommended of using them for general medicinal purpose and especially for treat wounds and burns diseases. It is strongly recommended of using them for production of effective pharmaceutical compounds and can be used as natural products of antimicrobial to treat wounds and burns diseases instead of chemical drugs. It is noticeable that the leaves of *prunus dulcis* are very rich in antioxidant content and therefore are good sources and safe and cheap for that.

CONFLICT OF INTEREST

"No conflict of interest associated with this work".

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Table 1: Qualitative tests for Phytochemical screening.

Phytochemical	Test Procedure
Alkaloids	In a test tube, 2-3 drops of Dragendorff's reagent was added to 0.1 ml of the extract orange precipitate indicated the presence of alkaloids. Dragendorff's test.
Terpenoids	In a test tube 5ml of extract was mixed in 2 ml of chloroform and then 3ml of concentrated sulfuric acid was added to form a layer. A reddish brown coloration forms at interface. Salkowski test.
Glycosides	Concentrated sulfuric acid in a test tube and extract sample were mixed with glacial acetic acid containing 1 drop of Ferric chloride (1:1:1volume). A brown ring appears in the presence of glycosides. Keller-Killani test.
Resins	To 5ml extract 5ml distilled water was added, the occurrence of turbidity shows the presence of resins. Turbidity test.
Saponins	To 5ml extract 5ml distilled water was added, the occurrence of turbidity shows the presence of resins. Foam test.
Tannins	A 4 ml extract was treated with 4 ml $FeCl_3$, the formation of green colour was taken as positive for tannin. $Fecl_3$ test.
Flavonoids	Extract was mixed with magnesium ribbon fragments, and concentrated hydrochloric acid was added drop wise. Orange, red, pink, or purple coloration indicates the presence of flavonoids. Shinoda test.
Phenols	Extract was mixed with 2 ml of 2% solution of FeCl3. A blue-green or black coloration indicated the presence of phenols. $Fecl_3$ test.
Amino acids	Extracts and 1 drop 2% Copper sulphate solution and 1 ml 95% ethanol excess of potassium hydroxide were mixed. Pink or yellow color in ethanol layer appears Biuret test.

Table 2: R_f values of TLC solvent system for different extracts of *Prunus dulcis*.

Phytochemical	Mobile phase	Confirmatory test	Extract	R_F Value
Alkaloids	Acetone:water:26% ammonia (90:7:3)	Dragendorff reagent	1 ml HCL+9 ml water	0.96
Flavonoides	Chloroform: Ethyl acetate (6:4)	Aluminum chloride reagent	70% ethanol	0.97
Tannins	Chloroform: Ethyl acetate (6:4)	10% $FeCl_3$ reagent	25ml water	0.99
Phenols	Toluene: Acetone: Formic acid (60:60:10)	10% KOH reagent	Methanol	0.97
Saponins	Ethyl acetate	Vanillin sulfuric acid reagent	Methanol	0.99

Table 3: Yields of *Prunus dulcis* leaves extracts from Methanolic and Aqueous extracts

M	Powder of plants	Amount of samples used (g)	Solvent	Volume of the solvent used (ml)	Extract yield/ (g)*
1-	*Prunus dulcis*	100	Pure Methanol	400	28.65±0.07
2-	*Prunus dulcis*	100	Distilled water	400	25.33±0.06

Mean values of the yield are presented as mean ± SEM. Values are statistically significant when $p \leq 0.05$.

Table 4: Phytochemical composition of the methanolic and aqueous Leaves Extracts of *Prunus dulcis.*

Plant	***Prunus dulcis***								
Chemical Compounds/ Solvents	Alkaloids	Terpenoids	Glycosides	Resins	Saponins	Tannins	Flavonoids	Phenols	Amino acids
Methanolic extract	+	+	+	+	+	+	+	+	+
Aqueous extract	+	+	-	+	+	+	+	+	-

Absence **(+)** Presence **(-)**.

Table 5: Antimicrobial activity of standard antibiotics discs against tested bacterial and fungal.

	Inhibition zones diameter (mm) of tested antibiotic				
Antibiotic	**AM(10ug)**	**CIP(25ug)**	**CF(30ug)**	**PZ (75ug)**	**PC(100ug)**
Organisms					
Staphylococcus aureus	19	26	20	21	20
Escherichia coli.	17	28	18	20	19
Pseudomonas aeruginosa.	18	30	17	21	18
Klebsiella sp.	20	33	22	23	17
Candida albicans	21	31	20	19	22

AM=Amoxycillin.CIP= Ciprofloxacin. CF=cefazllin. PZ=Cefoperazone.PC=piperacillin.

Table 6: Antimicrobial activity of the methanolic extracts of leaves of (*Prunus dulcis*) and standard antibiotics discs against tested bacterial and fungal.

Organisms	**Zone of inhibition(mm) Antibiotic**						
	0.5g/ml	**1.0g/ml**	**AM(10ug)**	**CIP(25ug)**	**CF(30ug)**	**PZ(75ug)**	**PC(100ug)**
Staphylococcus aureus.	15	13	19	26	20	21	20
Escherichia coli.	14	14	17	28	18	20	19
Pseudomonas aeruginosa.	13	14	18	30	17	21	18
Klebsiella sp.	12	14	20	33	22	23	17
Candida albicans.	13	11	21	31	20	19	22

Table 7: Antimicrobial activity of the Aqueous extract of leaves (*Prunus dulcis*) and standard antibiotics discs against tested bacterial and fungual.

Organisms	**Zone of inhibition(mm) Antibiotic**						
	0.5g/ml	**1.0g/ml**	**AM(10ug)**	**CIP(25ug)**	**CF(30ug)**	**PZ(75ug)**	**PC(100ug)**
Staphylococcus aureus.	18	17	19	26	20	21	20
Escherichia coli.	17	16	17	28	18	20	19
Pseudomonas aeruginosa.	16	16	18	30	17	21	18
Klebsiella sp.	13	14	20	33	22	23	17
Candida albicans.	16	18	21	31	20	19	22

Table 8: Antioxidant activities of the selected extracts and L- ascorbic acid using the (DPPH) free radical-scavenging assay

Plants	**Antioxidant activity DPPH (g/ml)**
L- ascorbic acid	87.5
Prunus dulcis	85.5