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Evaluation of Antibiotic and Antioxidant Activity of *Morus nigra* (Black Mulberry) Extracts Against Soil Borne, Food Borne and Clinical Human Pathogens

Maria Akram Minhas,[1] Amina Begum,[1] Saiqa Hamid,[1] Muhammad Babar,[1] Rabia Ilyas,[1] Shaukat Ali,[1] Farooq Latif[2] and Saiqa Andleeb[1]*
[1]Microbial Biotechnology Laboratory, Department of Zoology,
University of Azad Jammu & Kashmir, Muzaffarabad, Pakistan
[2]Bio-Processing Biotechnology Division, National Institute for Biotechnology
and Genetic Engineering, Faisalabad, Pakistan

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Authors' Contribution
SA conceived, designed and supervised the study. SA wrote the article and S Ali helped in writing. MAM and AB isolated bacteria and determined antibiotic activity of *M. nigra*. SH and RI performed phytochemical screening and TLC bioautography. MB performed TLC and trace element detection. FL performed HPLC.

Key words
Morus nigra
HPLC
Food borne pathogens

ABSTRACT

The aim of the present study was to evaluate the antimicrobial and antioxidant activities of fruits extracts of *M. nigra* prepared in methanol, ethanol, acetone, chloroform and diethyl ether solvent. Significant growth inhibition of all tested bacteria was recorded by *M. nigra* extracts as compared to fungal microbes. The phytochemical analysis showed that glycosides, tannins, saponins, and alkaloids were present in all extracts of *M. nigra* whereas free amino acids were absent. It was observed that phenolic and flavonoid contents were higher in ethanolic and acetone extracts as compared to others. Thin layer chromatography was employed to enumerate the antioxidants and phytochemical constituents in most active solvent extracts. The compounds contained in these extracts over silica gel were also evaluated through bioautography.

INTRODUCTION

Mulberry belongs to the genus *Morus* of *Moraceae* family. Few species of *Morus* such as *Morus alba*, *Morus laevigata*, *Morus nigra* and *Morus indica*, evaluated as edible fruits. *Morus nigra* (Black mulberry) is widespread in Northern India, Pakistan and Iran, Studies have been reported on the nutritional potentials of mulberry species (Ercisli and Orhan, 2007). The fruit, roots and bark of mulberry extracts have been used to treat diabetes, anemia, hypotension and other diseases (Ozgen *et al*., 2009). *Morus nigra* is famous for its nutritional qualities and medicinal properties. Fruits have good impact on blood glucose level and control blood cancer (Ahmad *et al*., 1985). In Turkey the *M. nigra* fruits are used for treating mouth lesions. Ozgen *et al*. (2009) reported that red and black mulberries played important role in the food industry due to the presence of anthocyanins. The exudative, proliferative, and antipyretic activities of mostly *Morus* species have been documented (Jawad *et al*., 1988). Venkatesh and Chauhan (2008) illustrated the function of root juices of *Morus* in agglutination of blood and killing of worms.

The current study was designed to investigate the antibacterial, antifungal and antioxidant activities of *M. nigra* extracts. The second objective was to screen the trace metal phytochemical constituent of these extracts.

MATERIALS AND METHODS

Sample collection

The fruit of *M. nigra* were collected at its optimum maturity stage (end of July 2013) from different areas of the Muzaffarabad, brought to the Biotechnology laboratory, Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan. The fruits were rinsed with running tap water, dried with tissue papers and let them to dry under shaded area for 10 days at room temperature. The dried materials were crushed into powder and sealed in air tight sealing bags. The crude extracts were prepared through conventional solvent extraction method. The powder (20 g) was soaked separately in 100 ml of solvents with increasing order of polarity; methanol, ethanol, acetone, chloroform, and diethyl ether, respectively for 10 days at room temperature, and crude extract were used as stock extract with 200 mg/ml final concentration.

Trace metal analysis

Trace metals such as sodium (Na), calcium (Ca), iron (Fe), manganese (Mn), zinc (Zn), potassium (K) and magnesium (Mg) were detected in mg/g units by atomic absorption spectrophotometer and cross checked by inductively coupled plasma atomic emission spectrophotometer. Standard procedure was adopted for

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* Corresponding author: drsaiqa@gmail.com

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the acid digestion. Solid samples were digested in Aqua Regia (HCl: HNO_3 (3:1)) for 3 days.

Test microorganisms

In current investigation, human clinical samples (urine, pus, blood) and waste soil samples (milk waste, slaughter house waste, engine oil waste, and sludge soil waste) were collected from different localities of Muzaffarabad, Azad Jammu and Kashmir, Pakistan. Different bacterial and fungal pathogens were isolated and identified through staining technique, biochemical tests and using different growth media like selective (Mannitol salt agar), differential (MacConkey's agar), enrichment (Blood agar and Chocolate agar) etc. The identification was done from Department of Zoology, Government College University, Lahore, Pakistan.

Gram-positive cocci (*S. aureus, S. pyogene and S. epidermidis, Enterococcus faecalis, Micrococcus* luteus) Gram-negative cocci (*Neisseria spp.,*) Gram-negative rod (*P. aeruginosa, K. pneumonia, E. coli,* and *S. marcescens*), and fungus strains such as *Aspergillus niger* and *Rhizopus oryzae* were isolated from human clinical samples (urine, pus, blood) and waste soil samples (milk waste, slaughter house waste, engine oil waste, and sludge soil waste) (Table I) and identified (Awan *et al*., 2013; Muhammad Bashart, M. Phil. Thesis, 2013).

Agar well diffusion method

The antimicrobial activity of extracts of *M. nigra* was tested using agar well diffusion method (Rios *et al*., 1988). Nutrient agar (Oxide: CMOO3) and nutrient broth media (Oxide: CM1) were used for bacterial culture whereas subouraud dextrose agar (Oxide: CM0041) and subouraud dextrose broth medium (Oxide: CM0147) were used for fungal culture. The microorganisms were activated by inoculating a loop full of strain in 25 ml of nutrient broth medium and incubated at 37°C on a rotary shaker for 24 h. The overnight culture was mixed with freshly prepared nutrient agar medium (NAM) at 45°C and was poured into the sterilized Petri dishes. All Petri dishes were kept at room temperature in laminar flow for solidification. In each plate, three wells of 5 mm diameter were made using a 1 ml of sterilized micropipette tip and sterilized needle was used for the removal of agar plug. Approximately 30 µl of each crude extract and control solvent samples were placed in each prepared wells and placed at 37^oC for 24-48 h. Microbial growth was determined by measuring the diameter of zone of inhibition after 24 h in millimeter (Seeley *et al*., 2001). Diameter of the clear zones (if greater then 5mm) around each well was measured with the help of scale (Hammer *et al*., 1999). Wells with ethanol, methanol, acetone, diethyl ether and chloroform were used as negative control. The results of sensitivity tests were denoted as (0-<1 mm) for no sensitivity, *(1-10 mm) for low sensitivity, **(11-19 mm) for moderate sensitivity and ***(20-35 mm) for high sensitivity.

Antibiogram analysis

Sensitivity test of various groups of antibiotics such as aminoglycosides (Streptomycin 10 µg/ml), Kanamycin 10 µg/ml, Penicillins (Ampicillin 10 µg/ml, Penicillin G 10 µg/ml), Tetracyclines (Tetracycline 10 µg/ml), and Fluoroquinolones (Ciprofloxacin 10 µg/ml, Nalidixic acid 5 µg/ml) and Chloramhenicol 10 µg/ml were tested against bacterial strains was assessed by agar disc diffusion method and used as positive control (Prescott *et al*., 1999; Bauer *et al*., 1996). The comparison of antibacterial activity of solvent extracts with that of standard antibiotics was also determined by activity index using following formula was used {Activity index= (zone of inhibition of extract/zone of inhibition of antibiotic)} (Shekhawat and Vijayvergia, 2010). On the other hand, antifungal index was measured by following formula; DC-DT/DC×100.

Free radical scavenging activity

Diphenyl-(2,4,6-trinitrophenyl)iminoazanium (DPPH) free radical scavenging activity of *M. nigra* extracts was evaluated by the method of You *et al.* (2006). Total antioxidant contents (TAC) was calculated as: %=[(Ao-Ai)/Ao]*100; where Ao is the absorbance of control without extract and Ai is the absorbance of the tested samples with the presence of extracts. The antioxidant constituents were also determined using thin layer chromatography (TLC) followed by DPPH technique (Moore *et al*., 2006). The antioxidant constituents were detected as yellowish green spots produced via bleaching of DPPH on the TLC plates.

Phytochemical screening

All extracts of *M. nigra* were screened for the presence of active phytochemical constituents such as glycosides, alkaloids, phenols, carbohydrates, proteins, flavonoids, tannins, and terpenoids (Sofowora, 1994; Harborne, 1998; Iyengar, 1985; Siddiqui and Ali, 1997; Trease and Evans, 2002). Folin–Ciocalteu reagent method was used to calculate total phenolic contents (TPC) with slight modifications (Zhou and Yu, 2006) and it was expressed as mg/g gallic acid equivalent (GAE) on the basis of calibration curve: y = 0.476x + 0.8, R2 = 0.996, where x was the absorbance and y was the gallic acid equivalent (mg/g). Total flavonoid contents (TFC) of extracts were quantified by the method illustrated by Zou *et al.* (2004) and it was expressed as mg/g rutin equivalent (RE) on the basis of calibration curve: y =

0.333x + 0.069, R2 = 0.999, where x was the absorbance and y was the rutin equivalent (mg/g). For flavonoids, spray TLC-developed plates with a 1% ethanolic/ methanolic solution of aluminum chloride and incubated for 10 min at room temperature. Yellow, brown, and dark green fluorescence in long wavelength UV light (360 nm) indicated positive results.

High performance liquid chromatography

Sugars and organic acids were detected through High performance liquid chromatography using modified protocol of Latif and Rajoka (2001). Glucose, xylose, ethanol, acetate, glycerol, xylitol, cellobiose and oligosaccharides were analysed by HPLC (Perkin Elmer, Norwalk, Connecticut, USA). Aminex HPX-87H column (300 x 7:8 mm^2: Bio-Rad, Richmond, California) was maintained at 45°C in a column oven. Sulphuric acid (0.001 N) in HPLC grade water at 0.6 ml/min was used as a mobile phase. The components were detected by refractive index detector and quantities using Turbochrom workstation software provided by the suppliers. All the chemicals were of HPLC grade.

Chromatogram development

The presence of major phytochemicals was further confirmed *via* thin layer chromatography (TLC) using Silica gel 60F264 plates (Wagner and Bladt, 2004). Ethyl acetate: butanol: distilled water (2:4:4) screening system were used. Ultra violet light (254-336 nm) was used to visualize bands on TLC developed plates. Retention factor (Rf) value of each spot was calculated as: Rf = distance travelled by the solute/distance travelled by the solvent.

TLC-Bioautography

For TLC-bioautography, agar overlay assay was used with few adjustments as illustrated by Slusarenko *et al*. (1998). TLC-Spot screening was performed using modified protocol of Joshi *et al.* (2011). Spots on the preparative silica gel plates were scratched with the help of clean and dry spatula and collected in beaker containing 70% ethanol and left overnight. The content in the beaker was stirred and filtrated and the filtrate containing active compound was used for the determination of antimicrobial effect.

Statistical analysis

Each experiment was repeated in triplicates and Standard Deviation from absolute data was calculated (http://easycalculation.com/statistics/standard-deviation.php). The comparison of antibacterial activity of solvent extracts with that of standard antibiotics was also determined by activity index (AI). For measuring activity index following formula was used {Activity index= (zone of inhibition of extract / zone of inhibition of antibiotic)}.

RESULTS AND DISCUSSION

Trace metals

Potassium was found in the greatest amount and averaged 1300 mg/g. Other minerals such as calcium (1000 mg/g), magnesium (1000 mg/g), sodium (55 mg/g), iron (68 mg/g), manganese (72 mg/g) and zinc (100 mg/g) were also determined. Trace metals such as iron, calcium and zinc are very important for health (Ercisli and Organ, 2007) and could help to improve the micronutrient status in children. *M. nigra* is readily available and very cheap (Nouman *et al*., 2011).

Antimicrobial activity

Yigit and Yigit (2009) had determined the antibacterial activities of methanol and aqueous extracts of *M. nigra* fruits and leaves against bacterial pathogens such as *E. coli, S. aureus, P. auroginosa* and *Proteus mirabilis* by disc diffusion method. In current research, as compared to non-polar solvents, polar solvents showed significant inhibition of infectious pathogens. The zones of growth inhibition against various bacterial and fungal pathogens were measured in millimeter (mm). Inhibited zones were found against all tested bacterial pathogens except fungal microbes, indicating the effective use of *M. nigra* extracts as antimicrobial agents (Table I). It was observed that both ethanolic and acetone extracts showed maximum inhibition of *E. coli* (20.0±0.0 mm and 20.0±0.0 mm), *S. aureus* (20.0±0.0 mm and 20.0±0.0 mm), *S. aureus (c2)* (22.0±0.0 mm and 20.0±0.0 mm), and *Neisseria* spp., (d1) (22.0±0.0 mm and 20.0±0.0 mm), respectively. Our results were consistent with Mazzimba *et al*. (2011) and Kuete *et al*. (2009). They represented the antibacterial activity of stem bark and stem wood of *M. nigra* against *S. aureus, B. subtillus, S. facials* and *P. aeroginosa.* Similarly, methanolic extract of *M. nigra* indicated the maximum inhibition of *K. pneumoniae* and *Neisseria* spp., (d1) with 22.0±0.0 mm and 23.0±0.0 mm zone of inhibition. Among the non-polar solvents, chloroform extract showed the maximum inhibition of *S. marcesscens, S. epidermidis, P. aeruginosa,* and *S. aureus* (25.0±0.0 mm, 23.0±0.0 mm, 25.0±0.0 mm, and 25.0±0.0 mm) whereas moderate effect was recorded against *S. pyogenes (C1)* with 15.0±0.0 mm zone of inhibition. On the other hand, diethyl ether extracts had low or no effect against all tested bacterial pathogens. It was noted that all extracts of *M. nigra* had no effect on fungal pathogens (Table I). Whereas the strong anticandidal activities of black mulberry fruits against some *Candida* spp. were reported

Table I.- **Zone of inhibition of extracts of *Morus nigra* against bacterial and fungal pathogens.**

Source of isolation		Pathogens	Zone of inhibition of extracts of *M. nigra* (M±SD) in mm				
			Methanol pH 6.85	**Ethanol pH 6.32**	**Acetone pH 5.86**	**Diethyl ether pH 4.86**	**Chloroform pH 5.59**
Clinical samples	Bacteria	*Escherichia coli*	1.7±0.0*	20.0±0.0***	20.0±0.0***	0.0±0.0	0.0±0.0
		Serratia marcesscens	15.0±0.0**	15.0±0.0**	20.0±0.0***	0.0±0.0	25.0±0.0***
		Klebsiella pneumoniae	22.0±0.0***	20.0±0.0***	1.4 ±0.0*	0.0±0.0	0.54±0.0
		Staphylococcus epidermidis	15.0±0.0**	15.0±0.0**	1.7±0.0*	0.0±0.0	23.0±0.0***
		Streptococcus pyogenes	10.0±0.0*	10.0±0.0*	5.0±0.0*	0.0±0.0	5.0±0.0*
		Pseudomonas aeruginosa	15.0±0.0**	15.0±0.0**	15.0±0.0**	0.0±0.0	25.0±0.0***
		Staphylococcus aureus	15.0±0.0**	20.0±0.0***	20.0±0.0***	0.0±0.0	25.0±0.0***
Engine oil soil		*Streptococcus pyogenes (e1)*	11.0±0.0**	16.0±0.0**	18.0±0.0**	0.0±0.0	0.0±0.0
		Micrococcus luteus (b1)	0.0±0.0	14.0±0.0**	17.0±0.0**	0.0±0.0	0.0±0.0
Slaughter house soil		*Enterococcus faecalis (a1)*	10.0±0.0*	11.0±0.0**	15.0±0.0**	0.0±0.0	10.0±0.0
Slaughter house soil		*Streptococcus pyogenes (C1)*	8.0±0.0*	10.0±0.0*	14.0±0.0**	5.0±0.0*	15.0±0.0**
Engine oil soil		*Staphylococcus aureus (c2)*	12.0±0.0**	22.0±0.0***	20.0±0.0***	8.0±0.0*	10.0±0.0*
Dairy waste		*Streptococcus pyogenes (H2)*	13.0±0.0**	11.0±0.0**	19.0±0.0**	2.0±0.0*	6.0±0.0*
		Streptococcus pyogenes (f2)	14.0±0.0**	20.0±0.0***	17.0±0.0**	3.0±0.0*	0.0±0.0
		Escherichia coli (H1)	18.0±0.0**	12.0±0.0**	14.0±0.0**	4.0±0.0*	5.0±0.0*
Sludge soil		*Neisseria spp.,* (d1)	23.0±0.0***	22.0±0.0***	20.0±0.0***	0.0±0.0	10.0±0.0*
Dairy waste	Fungi	*Aspergillus niger*	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Dairy waste		*Rhizopus oryzae*	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

(M±SD) Mean±Standard Deviation. Growth inhibition was expressed as (0) for no sensitivity, *(1- 10 mm) for low sensitivity, **(11-19 mm) for moderate sensitivity and ***(20-35 mm) for high sensitivity.

(Yiğit *et al.*, 2007). TLC-bioautography of *M. nigra* extracts supported the results of antimicrobial activity obtained through agar well diffusion method. Previous literature supported the antimicrobial and anti-inflammation activities of *Morus nigra* (Butt *et al.*, 2008).

Antibiogram analysis

Among all the antibiotics ciprofloxacin showed the maximum inhibition of almost all tested bacterial pathogens as indicated in Table II. Kanamycin indicated the greatest inhibited zone of *K. pneumoniae* and *S. epidermidis* (30.0±0.0 mm and 30.0±0.0 mm), while moderate inhibition of *S. pyogenes, S. marcesscens, E. coli, P. aeruginosa, S. aureus, M. luteus (b1), S. pyogenes (f2)* and *Neisseria spp*. Similarly, Streptomycin showed the maximum inhibition of *S. pyogenes, P. aeruginosa,* and *S. aureus* (25.0±0.0mm, 30.0±0.0 mm, and 33.0±0.0 mm) followed by Chloramphenicol with 20.0±0.0mm, 27.0±0.0 mm, and 29.0±0.0 mm zone of inhibition (Table II). The results also revealed that Tetracycline had moderate effect on *K. pneumoniae* (14.0±0.0) and *M. luteus* (12.0±0.0 mm), whereas low or no effect was recorded against other tested pathogens. On the other hand, Amoxilline, Tetracycline, and Penicillin G had no effect on *S. aureus.* Similarly, Tetracycline had no effect on *S. marcescens* and Ampicillin did not show such effect against all tested bacterial pathogens. Nystatin showed the moderate inhibition of *A. niger* and *R. oryzae* with 12.0±0.0 mm and 13.0±0.0 mm zone of inhibition. Recorded zones of inhibition of antibiotics were in agreement with the findings of Nouman *et al*. (2011). Fruit extracts of *M. nigra*, in comparison with the sensitivity of used standard antibiotics generally produced smaller zone of inhibitions. However, *M. nigra* extracts has advantage over the tested antibiotics because bacterial and fungal microbes have not yet developed resistant against it. It is due to the presence of higher amount of phytochemical constituents.

Antioxidant activity

In the present study the antioxidant activity of extracts of *M. nigra* fruits were determined by DPPH method in which radical scavenging activity by measuring the absorbance at 517 nm. The highest antioxidants were found in all used polar solvent extracts such as methanol> ethanol> acetone (100%, 97% and 96%). Our findings revealed with the previous study as demonstrated by Gerasopaulos and Stavroulakis (1997) that polar extracts *viz,* methanol, aqueous and methanol aqueous of *M. nigra* had 95%, 80% and 85% antioxidant activity, While low antioxidants were observed in non polar solvent extracts *i.e.*, diethyl ether >chloroform with

Table II.- **Zone of inhibition of antibiotics against bacterial and fungal pathogens.**

Pathogens	Zone of inhibition of antibiotics (M±SD) in mm							
	Bacterial antibiotics							Fungal antibiotic
	Streptomycin	Kanamycin	Ampicillin	Penicillin G	Tetracycline	Ciprofloxacin	Chloramhenicol	Nystatin
Escherichia coli	15.0±0.0**	12.0±0.0**	0.0±0.0	0.0±0.0	0.0±0.0	20.0±0.0***	15.0±0.0**	
Serratia marcesscens	29.0±0.0***	16.0±0.0**	6.0±0.0*	8.0±0.0*	0.0±0.0	26.0±0.0***	24.0±0.0***	
Klebsiella pneumoniae	33.0±0.0***	30.0±0.0***	6.0±0.0*	0.0±0.0	14.0±0.0**	33.0±0.0***	33.0±0.0***	
Staphylococcus epidermidis	15.0±0.0**	32.0±0.0***	2.0±0.0*	0.0±0.0	10.0±0.0*	33.0±0.0***	32.0±0.0***	
Streptococcus pyogenes	25.0±0.0***	11.0±0.0**	0.0±0.0	0.0±0.0	0.0±0.0	17.0±0.0**	20.0±0.0***	
Pseudomonas aeruginosa	30.0±0.0***	13.0±0.0**	6.0±0.0*	6.0±0.0*	6.0±0.0*	32.0±0.0***	27.0±0.0***	
Staphylococcus aureus	33.0±0.0***	16.0±0.0**	0.0±0.0	0.0±0.0	0.0±0.0	33.0±0.0***	29.0±0.0***	
Streptococcus pyogenes (e1)	17.0±0.0**	7.0±0.0*	0.0±0.0	0.0±0.0	0.0±0.0	16.0±0.0**	7.0±0.0*	
Micrococcus luteus (b1)	18.0±0.0**	18.0±0.0**	0.0±0.0	0.0±0.0	12.0±0.0**	30.0±0.0***	21.0±0.0***	
Enterococcus faecalis (a1)	13.0±0.0**	8.0±0.0*	5.0±0.0*	3.0±0.0*	3.0±0.0*	28.0±0.0***	18.0±0.0**	
Streptococcus pyogenes (C1)	14.0±0.0**	10.0±0.0	0.0±0.0	0.0±0.0	4.0±0.0*	20.0±0.0***	11.0±0.0**	
Staphylococcus aureus (c2)	15.0±0.0**	7.0±0.0*	0.0±0.0	0.0±0.0	0.0±0.0	25.0±0.0***	16.0±0.0**	
Streptococcus pyogenes (H2)	17.0±0.0**	6.0±0.0*	0.0±0.0	0.0±0.0	0.0±0.0	18.0±0.0**	5.0±0.0*	
Streptococcus pyogenes (f2)	25.0±0.0***	12.0±0.0**	0.0±0.0	0.0±0.0	0.0±0.0	21.0±0.0***	21.0±0.0***	
Escherichia coli (H1)	19.0±0.0**	7.0±0.0*	0.0±0.0	0.0±0.0	0.0±0.0	19.0±0.0**	7.0±0.0*	
Neisseria spp., (d1)	15.0±0.0**	17.0±0.0**	0.0±0.0	0.0±0.0	0.0±0.0	28.0±0.0***	10.0±0.0*	
Aspergillus niger								12.0±0.0**
Rhizopus oryzae								13.0±0.0*

(M±SD) Mean±Standard Deviation. Growth inhibition wasexpressed as (0) for no sensitivity, *(1- 10 mm) for low sensitivity, **(11-19 mm) for moderate sensitivity and ***(20-35 mm) for high sensitivity.

19% and 11% (Fig. 1A). Many previous studies reported that methanol and ethanol have been extensively used to extract antioxidant compounds from various fruits and vegetables such as strawberry, mulberry, sweet cherry, pomegranate and citrus peel (Pawlowska *et al*., 2008; Ercisli and Orhan, 2008; Dkhil *et al*., 2015). This finding has been confirmed through the 0.05 DDPH spray on TLC-developed plates. The yellow appearance of color indicated the presence of antioxidant agents in the *M. nigra* extracts (Fig. 1B). Antioxidant compounds mainly originated from medical plants have positive effect on health. Nadri *et al.* (2004) reported the antioxidant activity of three extracts of *M. nigra*. Mulberry juice has scavenging properties against superoxide, hydroxyl and nitric acid as indicated by Sakagami *et al*. (2006) and Sakagami *et al*. (2007).

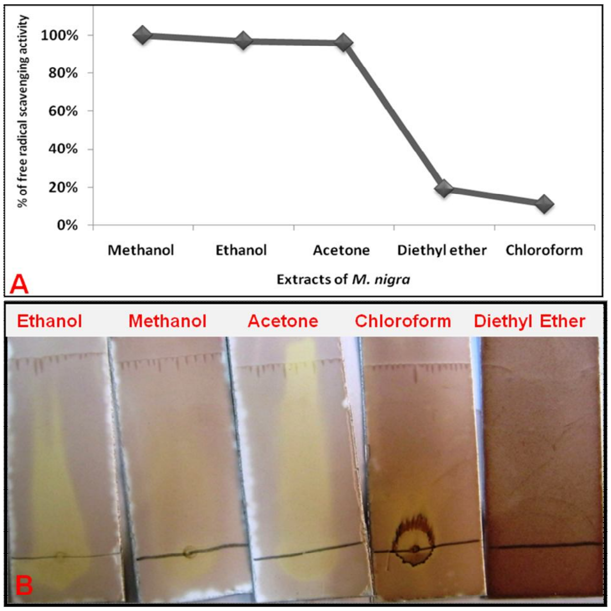

Fig. 1. Evaluation of antioxidant activity in *Morus nigra* extracts. A, Antioxidant analysis through DPPH free radical scavenging methods; B, Antioxidant constituent evaluation on TLC-developed plates through 0.05% DPPH sprayed method.

Phytochemical constituents

Current study revealed the presence of bioactive constituents in fruit extracts of *M. nigra*. Phytochemical analysis showed that tannins, Saponins, alkaloids and glycosides were present in all fruit extracts of *M. nigra* whereas terpenoides and phlobatannins were found in methanol, ethanol, chloroform, and acetone extracts of *M. nigra*. Amino acids test did not show purple color therefore, it was indicated the absence of free amino acids. On the other hand, carbohydrates and proteins test showed the presence methanol, ethanol, and acetone, whereas not found in chloroform and diethyl ether. The data obtained from the HPLC quantification of sugars from *M. nigra* extracts revealed that oligo's, glucose and xylose were the predominant sugars at 6.44, 9.79 and 10.53 nm in mulberry. On the other hand, minute quantity of oligo's was found in both diethyl ether and ethanol extracts. Acetone and diethyl ether extracts indicated the presence of greater amount of xylose compared to glucose. Cellubiose was only detected in diethyl ether extracts. Acetone extracts indicated the presence of organic acids such as acetic acid, ascorbic acids, succinic acid and lactic acid as major components of mulberry fruit (Fig. 2). The presence of ascorbic acids in *M. nigra* extracts were also evaluated by various researchers (Iqbal *et al*., 2010; Ercisli *et al*., 2010).

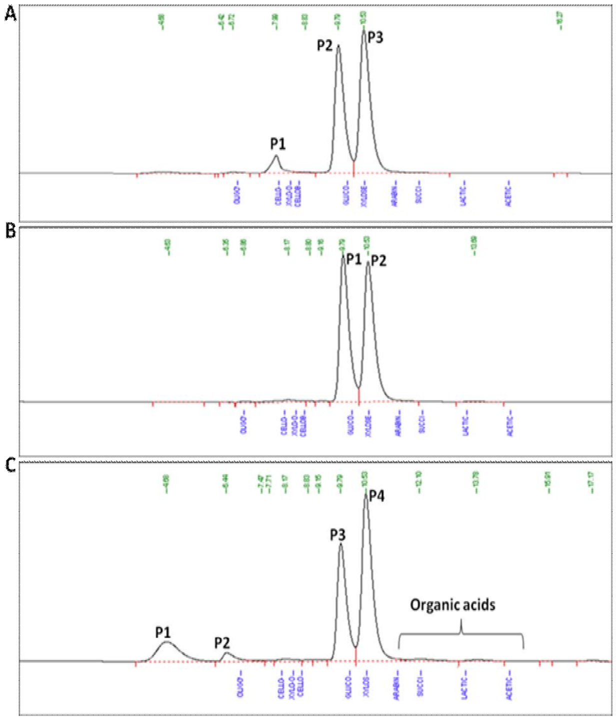

Fig. 2. HPLC of *Morus nigra* extracts. A: Diethyl ether extract of *M. nigra;* Three different peaks were observed, P2 and P3 indicated the presences of glucose and xylose sugars whereas P1 indicated the presence of cellubiose. **B:** Ethanol extract of *M. nigra*; Two peaks indicated the presences of glucose and xylose sugars. **C:** Acetone extract of *M. nigra;* Four different peaks observed, P2, P3, and P4 indicated the presences of oligo's, glucose and xylose sugars. P1 is unknown peak when compared with standards.

These results also confirmed the presence of phenolics and flavonoids in fruit extracts of *M. nigra.* Combination of different organic-aqueous solvents indicated the efficient recovery of polyphenolics from the extracts of medicinal plants. Similar results were shown by Ozgen *et al*. (2009). They reported that maximum phenolic compounds from *Morus nigra* and *Morus rubra* were obtained with mixtures of acetone, water, and acetic acid (70:29.5:0.5). The total phenolic contents of *M. nigra* extracts were 2.055 mg/g as gallic acid equivalent. It was consistent with the results of Ercisli *et al*. (2010). The range of TPC in mulberry was 1.5 to 2.57 mg/g according to the Hojjatpanah *et al*. (2011). It was reported that black mulberry fruit contains high amount of total phenolics, total flavonoid and ascorbic acid (Hassimotto *et al*., 2007; Nitra *et al*., 2007). Overall, the results of phytochemical study reveal that the *M. nigra* extracts are a potential source of natural sugars, organic acids, minerals, antioxidants and antimicrobials particularly at ripened stage.

CONCLUSION

The current research demonstrated the antimicrobial activity of *M. nigra* extracts against both Gram-positive and Gram-negative bacteria as well as fungal microbes. High phenolic contents and antioxidants promote the antimicrobial activity of *M. nigra*. *M. nigra* may be an effective antibiotic against various bacterial and fungal pathogens.

Statement of conflict of interest

Authors have declared no conflict of interest.

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