

Evaluation of Wood, Bark and Leaves Extracts from *Maclura pomifera* (Rafin.) Schneider (Moraceae) Against the Growth of Some Pathogenic Bacteria

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In the present study, a different solvent extract of wood, bark and leaves from *Maclura pomifera* were tested for their antibacterial activities against the growth of four human pathogenic bacteria namely; *Micrococcus luteus*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. The test was evaluated by means of disc-diffusion method at extract concentration of 2000 µg/mL. The bacterial strains *M. luteus* was inhibited best by wood and bark ethanol extract (percent of inhibition (PI) value of 86.96%), *S. aureus* inhibited significantly by bark chloroform extract (PI value of 61.90%), *A. baumannii* strongly inhibited by bark ethanol extract (PI value of 100%) followed by bark chloroform extract (PI value of 65%), and *P. aeruginosa* was moderately inhibited by the leaves chloroform extract (PI value of 53.85%). The present investigation showed the potential activity of extracts from *M. pomifera* for controlling the infectious diseases caused by human bacterial pathogens.

Key words: *Maclura pomifera*; Pathogenic bacteria; Extracts; Wood; Bark; Leaves.

Osage orange; *Maclura pomifera* (Rafin.) Schneider (syn. *Maclura aurantiaca* Nutt., *Ioxylon pomiferum* Raf., *Toxylon pomiferum* Raf. Ex Sarg.), a member of Moraceae family, is a small to medium size tree 36 to 65 feet tall, with deeply furrowed bark and thorny branches and widely cultivated hardwood tree for ornamental purposes (Salem and Mohamed, 2013; Kupeli *et al.*, 2006). Yellow dye can be extracted from the root bark (Wolfrom and Bhat, 1965), and the macerated roots were used to treat of sore eyes by Comanche Indians in the

North America (Carlson and Volney, 1940). In Bolivia, leaves and bark are used for uterine haemorrhage (Bourdy *et al.*, 2004) and the bark has been reported for using against toothache (Bueno *et al.*, 2005). Wood of *M. pomifera* is being resistive to oxidative sine the wood have many chemical which are responsible for preventing the decay by bacterial and fungal as well as insect attacks, making it an ideal building material (Clopton and Roberts, 1949; Smith and Perino, 1981; Monache *et al.*, 1995).

Chemically, heartwood was reported to have 2',3,4',5,7, pentahydroxyflavone and was responsible for the resistance against the fungi attack (Barnes and Gerber, 1955) as well as flavones and xanthenes (Wolfrom and Bhat, 1965). Lectins,

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triterpenes, xanthenes and flavone-type compounds (Gerber, 1986; Young *et al.*, 1995; Lee *et al.*, 1998; Marek *et al.*, 2003) were also reported. Many studies were reported the activity of extracts and its components from *M. pomifera* as antibacterial, antioxidant, antifungal, antiviral, cytotoxic, antitumor, estrogenic and antimalarial activities agents (Hay *et al.*, 2004; Bunyapraphatsara *et al.*, 2000; Maier *et al.*, 1995; Mahmoud, 1981) which has been attributed to flavonoid components (isoflavones, osajin, pomiferin and dihydroxychalcon derivative) (Vesela *et al.*, 2004; Cioffi *et al.*, 2003; Tsao *et al.*, 2003).

Additionally, the heartwood was reported to contain the pigments morin, oxyresveratrol (2,3',4,5'-tetrahydroxystilbene), and 1,3,6,7-tetrahydroxyxanthone, and oxyresveratrol was observed remarkable fungicidal and termiticidal agents (Wolfrom and Bhat, 1965; Barnes and Gerber, 1955). The anti-inflammatory and antinociceptive activity of *M. pomifera* and the two prenylated isoflavones; scandenone and auricularin was reported by Kupeli *et al.*, (2006). Also, xanthenes and flavanones were found in the root bark of the *M. pomifera* (Teixeira and da Costa, 2005). *M. pomifera* has been well-known for its rich phenolic content, especially prenylated isoflavonoids and the methanol extracts from the leaf, wood, flower, twig, and stem bark of the female and male have been showed anticholinesterase potential (Orhan *et al.*, 2009). Also, isoflavone levels have been recently determined in the fruits (Darji *et al.*, 2013).

In the present study different solvents extract (acetone, cold water, ethanol, and chloroform) from different parts (wood, bark, and leaves) of *Maclura pomifera* were used to evaluate their antibacterial activity against the growth of four human pathogenic bacteria namely; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Micrococcus luteus*.

MATERIALS AND METHODS

Plant Material

Different parts (wood, bark, and leaves) from *M. pomifera* were collected in August 2013 from Antoniadis Garden, Horticultural Research Institute, Alexandria, Egypt. The plant was kindly

identified and obtained voucher number at Egypt barcode of life project, Faculty of Agriculture, Alexandria University. All the materials (wood, bark and leaves) were air-dried at room temperature and ground into small particles with small laboratory mill.

Preparation of Extracts

Three groups from ground leaves (50 g from each of them) were macerated with 100 mL for each of acetone, chloroform, and ethanol. Three groups of fifty grams for each of them from the ground wood samples were macerated in 100 mL of each acetone, cold water, and chloroform. The ground bark samples were distributed for four groups, each of them 50 grams and macerated in 100 mL of chloroform, acetone, cold water, and ethanol. After one week of maceration, the extracted materials were filtrated through filter paper (Wattman No.1), and the residues were discarded (Ali *et al.*, 2014). All the extracts were concentrated under reduced pressure at 45°C using a rotary evaporator. The extracts were weighed and stored at 4°C in the refrigerator until further uses. For the bioassay, the extracts were prepared at a concentration of 2000 µg/mL by diluting the crude extract in 10% Dimethylsulfoxide (DMSO, Sigma-Aldrich, Germany) and distilled water (1:1 v/v) (Ali *et al.*, 2013; Ahmad and Sariri, 2008). In the present study we used eight different extracts and all the extracts at a concentration of 2000 µg/mL were stored in brown vials until the beginning of the bioassay.

Antibacterial Activity

The antibacterial activity of the extracts from wood, bark, and leaves of *M. pomifera* was achieved using the Kirby-Bauer disc diffusion method (Bauer *et al.*, 1996) against the following human pathogenic bacteria; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Micrococcus luteus* which were supplied by the Botany Department, Microbiology Section, Faculty of Science, Alexandria University, Egypt. Nutrient agar (NA) medium was used for maintenance of the tested bacterial organisms. Mueller Hinton agar (MHA) was used in all bioassays applying the disc diffusion method. Briefly, filter paper discs with 5 mm in diameter were loaded with 20 µL of 2000 µg/mL extract concentration and placed on the inoculated plates with the respective bacterium and

incubated at 37°C for 24 hrs. Tetracycline (20 µg/disc) was used as a positive control. Negative control discs were loaded by the respective solvents used [(DMSO and distilled water (1:1 v/v)]. The diameters of the inhibition zones were recorded for three replicates in millimeters (mean±SD).

Activity index and Percent of Inhibition

Activity Index (A.I.) and Percent of Inhibition (P.I.) were calculated for all solvent extracts obtained at a concentration of 2000 µg/ml using the following formula (Munazir *et al.*, 2012):

A.I.= mean IZ for each solvent / IZ obtained by the standard antibiotic

P.I. = Activity index × 100

RESULTS AND DISCUSSION

The quantities of the weighted extracts from the different parts (wood, bark, leaves) of *M. pomifera* with respect to the oven dry weight are presented in Table 1. The highest quantity was observed by the ethanol extract from the tree bark (12 g/100 g o.d. weight) followed by the cold water extract from bark (10.90 g extract/100 g o.d. weight) and the ethanol extract from the leaves (8.90 g extract/100 g o.d. weight), where the lowest quantity was shown by the acetone extract from the leaves (3.15 g extract/100 g o.d. s weight).

The antibacterial activities of the extracts are shown in Table 2. The results observed that the highest inhibition zones (IZs) found against the growth of *M. luteus* were present by the ethanol

extract from leaves and bark with IZ value of 20mm, followed by the chloroform extract of leaves and wood with IZ value of 16mm and acetone extract from bark with IZ value of 15mm. the moderate activity was shown by the chloroform extract from bark (IZ value of 12mm), bark cold water extract (IZ value 11mm) and leaves acetone extract (IZ value 10mm). On the other hand, the wood acetone and cold water extracts did not show any activity at a concentration of 2000 µg/mL.

The highest IZs found against the growth of *S. aureus* were observed by the bark chloroform and acetone extracts ((13mm, and 12mm, respectively), followed by bark ethanol extract (IZ, 11mm), and leaves chloroform extract (IZ, 11mm), while the lowest values were found by wood cold water (IZ, 8mm) and chloroform (IZ, 7mm) extracts. On the other hand, leaves (acetone, ethanol), wood (acetone) and bark (cold water) extracts did not show any IZs at the concentration of 2000 µg/mL.

Bark ethanol extract from the highest IZ against the growth of *A. baumannii* with a value of 20mm, followed by chloroform extract (IZ, 13mm). The moderate IZs values were observed by bark acetone extract (IZ, 11mm), leaves chloroform and acetone (IZ, 10mm), and wood chloroform (IZ, 10mm) extracts. Furthermore, the extracts of leaves and wood acetone, wood and bark cold water didn't show any activity against the growth of *A. baumannii* the concentration of 2000 µg/mL.

The best IZ observed against the growth of *P. aeruginosa* was found by the leaves chloroform extract (14mm), followed by the Cold water extract of wood (12mm). The moderate activity was reported by leaves, wood, and bark acetone extract (10, 9, and 10mm, respectively), chloroform (7mm) and ethanol (11) extract from the bark.

Ethanol extracts of different parts of *M. pomifera* have been reported for exhibiting antimicrobial and antifungal activity (Allen, 1985; Delle Monache *et al.*, 1984; Mahmoud, 1981). For example, Alcoholic fraction of *M. pomifera* fruit extract was showed interesting antimicrobial activity and osajin and pomiferin, were found to be responsible for the antimicrobial activity of the extract of its fruits (Mahmoud, 1981). Furthermore, the chloroform extract of stems and leaves has been shown to have an activity against a highly specific oestrogen-regulated transcription system cell

Table 1. The quantity of the weighted extracts from wood, bark and leaves of *M. pomifera*

Part	Extract	g extract/100 g oven dry weight
Leaves	Acetone extract	5.45
	Chloroform extract	4.12
	Ethanol extract	8.90
Wood	Acetone extract	3.15
	Cold water extract	7.35
	Chloroform extract	4.16
Bark	Chloroform extract	4.20
	Acetone extract	7.40
	Ethanol extract	12
	Cold water extract	10.90

cultures in transformed *Saccharomyces cerevisiae* (Maier *et al.*, 1995) and the activity was related to coumestan, isoflavones, flavones and lignans in other plants (Miksicek, 1993).

According to the AI and PI values presented in Table 3, the bacterial strains *M. luteus* was inhibited best by wood and bark ethanol extract (PI value of 86.96%), *S. aureus* inhibited significantly by bark chloroform extract (PI value of 61.90%), *A. baumannii* strongly inhibited by bark ethanol extract (PI value of 100%) followed by bark chloroform extract (PI value of 65%), and *P. aeruginosa* was moderately inhibited by the

leaves chloroform extract (PI value of 53.85%).

The antibacterial activity of extracts could be related to the previously reported triterpenes (lupeol, butyrospermol, and lupane-3 α ,20-diol), osajin and pomiferin, and 19 α -H-lupeol a epimer of lupeol (Gearien and Klein, 1975); isoflavones, osajin, pomiferin and dihydroxychalcon derivative (Vesela *et al.*, 2004; Cioffi *et al.*, 2003; Tsao *et al.*, 2003) and scandenone and auriculasin was reported by Kupeli *et al.*, (2006).

A number of extractives have been identified previously from wood, bark, fruit seed, and roots (Rowe *et al.*, 1979; Eperjessy and Elek,

Table 2. Antibacterial activity (inhibition zones in mm) of extracts from different parts of *M. pomifera* against the growth of four human pathogenic bacteria.

Part	Extract	Inhibition Zone (mm)			
		<i>M. luteus</i>	<i>S. aureus</i>	<i>A. baumannii</i>	<i>P. aeruginosa</i>
Leaves	Acetone extract	10 \pm 0.12	na	na	10
	Chloroform extract	16 \pm 0.14	11 \pm 0.8	10	14 \pm 0.30
	Ethanol extract	20 \pm 0.64	na	10	na
Wood	Acetone extract	na	na	na	9
	Cold water extract	na	8 \pm 0.66	na	12
	Chloroform extract	16 \pm 0.90	7	10	na
Bark	Chloroform extract	12 \pm 0.7	13	13 \pm 0.4	7
	Acetone extract	15 \pm 0.12	12	11	10
	Ethanol extract	20 \pm 0.14	11	20 \pm 0.14	11
	Cold water extract	11 \pm 0.10	na	na	na
Tetracycline*		23	21	20	26
DMSO		na	na	na	na

na: Not active.

Inhibition Zone (mm) including disc diameter of 5 mm at 2000 μ g/mL of the extract.

*: Positive control (Tetracycline 20 μ g/disc).

Table 3. Activity index and % of inhibition of extracts from different parts of *M. pomifera* against the growth of four human pathogenic bacteria.

Part	Extract	Bacterial strains							
		<i>M. luteus</i>		<i>S. aureus</i>		<i>A. baumannii</i>		<i>P. aeruginosa</i>	
		AI	PI%	AI	PI%	AI	PI%	AI	PI%
Leaves	Acetone extract	0.43	43.48	na	na	na	na	0.38	38.46
	Chloroform extract	0.70	69.57	0.52	52.38	0.50	50	0.54	53.85
	Ethanol extract	0.87	86.96	na	na	0.50	50	na	na
Wood	Acetone extract	na	na	na	na	na	na	0.35	34.62
	Cold water extract	na	na	0.38	38.10	na	na	0.46	46.15
	Chloroform extract	0.70	69.57	0.33	33.33	0.50	50	na	na
Bark	Chloroform extract	0.52	52.17	0.62	61.90	0.65	65	0.27	26.92
	Acetone extract	0.65	65.22	0.57	57.14	0.55	55	0.38	38.46
	Ethanol extract	0.87	86.96	0.52	52.38	1	100	0.42	42.31
	Cold water extract	0.48	47.83	na	na	na	na	na	na

1969; Wolfrom *et al.*, 1964; Jacobs and Morris; 1951), morin, oxyresveratrol (2,3',4,5'-tetrahydroxystilbene), and 1,3,6,7-tetrahydroxyxanthone, and oxyresveratrol was observed remarkable fungicidal and termiticidal agents (Wolfrom and Bhat, 1965; Barnes and Gerber, 1955). Also, heartwood has been reported to have a nontoxic antibiotic useful as a food preservative and an antifungal agent (Barnes and Gerber, 1955; Jacobs, 1951).

Moreover, the antibacterial activity could be related to the prenylated flavonoids (5,7,4',20-tetrahydroxy-6-[30-methyl-but-30-enyl]-flavone, 5,4'-dihydroxy-20-(1-hydroxy-1-methylethyl)-30-methoxyfurano(40,50(6,7) isoflavone and 5,4'-dihydroxy-20-(1-hydroxy-1-methylethyl)-30-methoxyfurano (40,50 (6,7) flavone), which have been isolated from the chloroform extracts of stems and leaves (Lee *et al.*, 1998).

CONCLUSIONS

The antibacterial activity of different solvent extracts from different parts (wood, bark, and leaves) of *M. pomifera* against the growth of four human pathogenic bacteria was reported. The bacterial strains *M. luteus* was inhibited best by wood and bark ethanol extract, *S. aureus* inhibited significantly by bark chloroform extract, *A. baumannii* strongly inhibited by bark ethanol extract followed by bark chloroform extract, and *P. aeruginosa* was moderately inhibited by the leaves chloroform extract. The present investigation showed the potential activity of extracts from *M. pomifera* for controlling the infectious diseases caused by human bacterial pathogens.

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