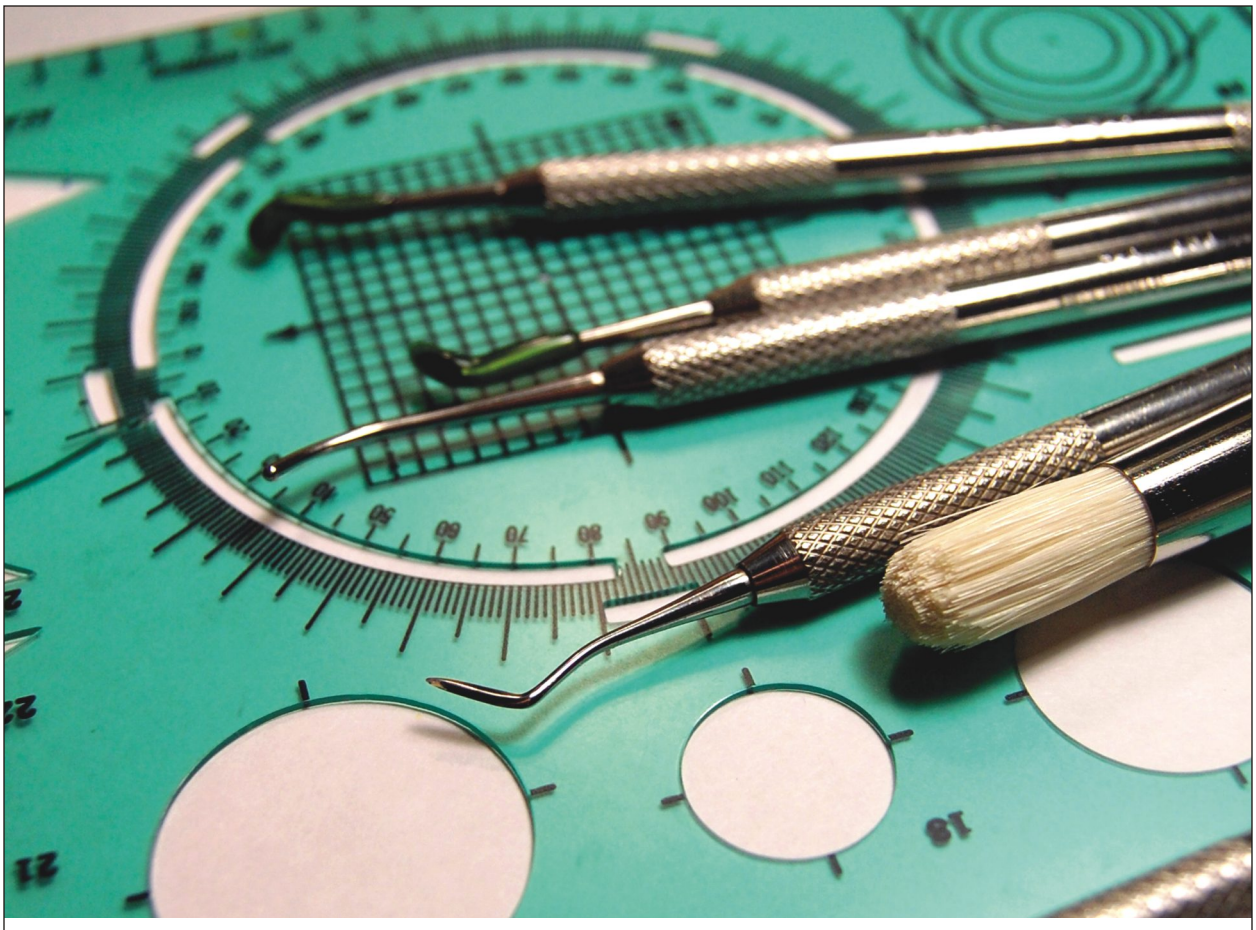

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Research Article
Effects of *Malus domestica* Fruit Extracts Against Clinically Isolated Dental Pathogens

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Abstract

Background and Objective: Medicinal plants have a vital role in the development of novel antimicrobial agents. The fruit *Malus domestica* is known as apple and commonly used to prevent tooth decay by killing dental pathogens. However, there were no scientific reports about the fruits of *Malus domestica* having antimicrobial activity against dental pathogens. The aim of this study was to extract the active constituents present in the fruits of *Malus domestica* by cold maceration method using petroleum ether, ethanol and water. **Methodology:** The antibacterial activity was assessed by cup plate method against five isolated dental pathogens such as *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus subtilis*, *Klebsiella* sp. and *Pseudomonas* sp. and the radial zone of inhibition was measured. **Results:** The results revealed that the ethanol extract (100 mg mL^{-1}) has more potent antibacterial activity against *S. aureus*, *S. mutans* and *Pseudomonas* sp., followed by aqueous extract (100 mg mL^{-1}) whereas, petroleum ether extract (100 mg mL^{-1}) has not shown any zone of inhibition against the tested microorganisms. All the results were compared with standard drug chlorhexidine 0.2% w/v. **Conclusion:** Hence, the study concludes that *Malus domestica* fruit extracts possess significant ($p<0.001$, $p<0.01$, $p<0.05$) antibacterial activity against dental pathogens. The phyto chemical tests on ethanol extract showed the presence of bioactive constituents such as terpenoids, flavonoids, phenolic compounds and tannins which could be responsible for the antibacterial activity of *M. domestica* fruit.

Key words: *Malus domestica* fruit, ethanol extract, dental pathogens, antibacterial, cup plate method

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tooth decay also known as dental caries is one of the most common infectious diseases throughout the world and lead to damage the hard tooth structure and causes infections. This is caused by the bacterium that lives in the mouth[1]. Bacteria, staphylococci and streptococci ferment sugars and produce acids. These acids affect primary decalcification of enamel which leads to total destruction and the decalcification of dentin. Major end products of fermentation are lactic acid, dextrans and levans which are found to cause dental caries[2,3].

In recent years many types of toothpaste, mouthwash and antibiotics are introduced, still tooth decay and its related complications continue to be a major medical problem. Over the last decade, the awareness about the ways to prevent the microbial infections has been increasing. Natural products are the fundamental source of the development of new pharmaceuticals search for novel antimicrobials from natural products against pathogens continues to discover more effective and less toxic antimicrobial agents[4]. Due to the identification of medicinal plants from indigenous pharmacopoeias and the recognition of the value of traditional medical systems, particularly of Asian origin, plants still make an important contribution to health care in modern medicine[5]. Medicinal plants produce a definite physiological action on the human body due to the presence of some secondary metabolites in the plant tissues which determines the medicinal value of plants[6].

Now a days, screening of antimicrobial activity of several medicinal plants has been increased due to the increasing failures of chemotherapeutic agents and antibiotic resistance exhibited by pathogenic microbes[7]. *Malus domestica* which belongs to the family, Rosaceae is commonly known as apple. The plant is widely distributed in China, United States, Iran, Turkey, Russia, Italy, Malaysia and India. The fruit of *Malus domestica* is traditionally used to prevent the tooth decay by killing the dental pathogens. The fruits are consumed worldwide in different forms such as fresh juices and cider[8]. Their beneficial properties to human health are related to the high content of phenolic compounds, dietary fibre, sugar and vitamins[9] which are responsible for curing cancer, cardiovascular diseases, asthma and diabetes[10].

Apples contain many types of phenolic derivatives and flavonoids (flavan-3-ols, flavonols, procyanidins, chalcones and anthocyanins). *Malus domestica* exhibit efficient antioxidant property owing to the presence of its phytoconstituents which are also well known to have anti-inflammatory, antiviral and antimicrobial properties[11]. However, there was no scientific report for the antimicrobial activity of *Malus domestica* fruits against dental pathogens. Hence the present work was undertaken to scientifically evaluate the antibacterial activity of *M. domestica* fruit against clinically isolated dental pathogens.

MATERIALS AND METHODS

Collection and authentication: The fruits of *M. domestica* (Fuji apple) were purchased from local market, Kota Seriemas, Nilai, Malaysia in the month of March, 2011. The collected fruits were authenticated by a Botanist at KPJ Healthcare University College, Kota Seriemas, Nilai, Malaysia.

Preparation of extracts: The purchased fruits *M. domestica* were washed thoroughly with water and sliced into small pieces and pulverized. The pulverized fruits (1 kg) were extracted successively with petroleum ether, ethanol and water by cold maceration technique for 7 days. The mixture was filtered separately and the excessive solvents were evaporated. The extracts were concentrated using Rotary vacuum evaporator under reduced pressure. The colour, consistency and percent yield of the extracts are depicted in Table 1. All the extracts were stored in desiccators until use[12].

Isolation of dental pathogens: Five pathogenic microorganisms viz., *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus subtilis*, *Klebsiella* sp. and *Pseudomonas* sp., were isolated from the dental cavities of 24 patients (6 males and 18 females) (Table 2). The isolates were identified by standard morphological analysis and bio-chemical tests, respectively[13]. The identity of the microorganisms was further confirmed by growing in specific media as per the method developed by Cheesborough[14].

Screening of antibacterial activity of various extracts against the isolated dental pathogens: The antibacterial activity of the extracts of *M. domestica* fruits was assessed by

Table 1: Colour, consistency and yield (%) of *Malus domestica* fruit extracts

Extracts	Colour	Consistency	Yield (%)
Petroleum ether	Yellowish	Slightly sticky	3.2
Ethanol	Dark brown	Semisolid	13.4
Water	Reddish brown	Semisolid	30.3

Table 2: Clinically isolated dental pathogens

Pathogens	Types	No. of isolates	Isolates (%)
Staphylococcus aureus	G (+ve)	4	17
Streptococcus mutans	G (+ve)	9	38
Lactobacillus subtilis	G (+ve)	8	33
Klebsiella sp.	G (-ve)	11	46
Pseudomonas sp.	G (-ve)	6	25

Table 3: Antibacterial activity of *M. domestica* fruit extracts against dental pathogens

	Zone of inhibition (mm)			
Bacteria	Petroleum ether extract	Ethanol extract	Aqueous extract	Chlorhexidine
Streptococcus mutans	No zone	16.7±1.8^{aaa}	15.7±0.6^{aaa}	18.1±0.3^{aaa}
Staphylococcus aureus	No zone	17.2±1.2^{aaa}	15.4±0.1^{aa}	17.8±1.5^{aaa}
Lactobacillus subtilis	No zone	8.5±1.5^{a}	8.9±0.1^{a}	11.8±1.6^{aa}
Klebsiella sp.	No zone	9.2±1.6^{a}	8.6±0.6^{a}	13.4±2.3^{aa}
Pseudomonas sp.	No zone	16.3±1.1^{aaa}	16.0±0.3^{aaa}	18.6±0.8^{aaa}

$^{a}p<0.05$, $^{aa}p<0.01$, $^{aaa}p<0.001$, radial zone of inhibition of *M. domestica* fruit extracts against bacteria vs. the normal diameter of cup (8 mm)

cup-plate method[15] with modifications. Twenty milliliters of the Muller Hinton agar media was poured into sterile petri dishes and left to solidify. The isolated bacterial suspensions (10^8 CFU mL^{-1}) were streaked uniformly in two directions at 90° by using sterile swabs on the surface of Muller Hinton agar medium. In each of these plates, 5 cups (8 mm in diameter) were made using a sterile cork borer. Cups were filled with 0.1 mL of each extract of *M. domestica* (100 mg mL^{-1}), standard drug, chlorhexidine (0.2% w/v)[16] and normal saline. The plates were then incubated in the upright position at 37°C for 18-24 h. After incubation, the radial zone of inhibition in millimetre was measured[17]. Each sample was assessed in triplicate and the data is expressed as Mean±SEM. The results of bacterial sensitivity testing on *M. domestica* fruit extracts were compared with reference standard and tabulated in Table 3.

Preliminary phytochemical analysis: The highly active extract of *M. domestica* fruits was subjected to preliminary phytochemical screening by standard methods to identify the presence of secondary metabolites such as alkaloids, amino acids, carbohydrates and glycosides, fixed oils and fats, flavones and flavanones, gums and mucilage, phenolic compounds and tannins, proteins, saponins, sterols and terpenoids[18]. The results are tabulated in Table 4.

Statistical analysis: The values are represented as Mean±SEM and the data obtained from this study was subjected to one-way analysis of variance (ANOVA) followed by Dunnett's t-test.

RESULTS

The percentage yield of extracts of *M. domestica* fruits was in the range of 3.2-30.3. Aqueous extract had the highest percentage yield followed by ethanol extract and showed the presence of more secondary metabolites which may be soluble in high polarity solvents. The pathogens, gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus subtilis*, Gram-negative bacteria such as *Klebsiella* sp. and *Pseudomonas* sp. were isolated and identified from 24 patients having dental cavities (Table 2).

Table 3 represents antibacterial activity of all the extracts of *M. domestica* fruits at concentration of 100 mg mL^{-1} against each of the isolated organism. Anti-bacterial activity against the isolated pathogens was assessed by cup plate agar diffusion method and the extracts showed varying degree of inhibitory effect (Table 3). The results showed that the ethanol extract (100 mg mL^{-1}) has more potent antibacterial activity against *S. aureus*, *S. mutans* and *Pseudomonas* sp., followed by aqueous extract (100 mg mL^{-1}) whereas, petroleum ether extract (100 mg mL^{-1}) has not shown any zone of inhibition against the tested microorganisms. The study revealed that the ethanol extract exhibited maximum zone of inhibition compared to the other extracts. All the results were compared with chlorhexidine 0.2% w/v. Also ethanol extract exhibited high significant ($p<0.001$) antibacterial activity against *S. aureus* than aqueous extract ($p<0.01$).

DISCUSSION

The earlier study by Ahmad *et al.*[19] supported the potent antibacterial activity of ethanol extract (100 mg mL^{-1}) than aqueous extract. Ahmad *et al.*[19] screened medicinal plants to detect antimicrobial activity and demonstrated that alcohol is a better solvent as compared to water. Successful prediction of bioactive compounds from plant material is largely dependent on the type of solvents used in the extraction procedure. The pure bioactive compound from active extract could be a good lead antibacterial compound which on further modification can be safe and effective antibiotic. Consequently for a continuous search of new effective and affordable antimicrobial drugs, phytochemical tests on active ethanol extract of *M. domestica* fruit was carried out which showed the presence of terpenoids, flavonoids, phenolic compounds and tannins (Table 4). The antibacterial activity may be due to the individual phytoconstituents present or in the combination.

Table 4: Preliminary phytochemical tests of active ethanol extract of *Malus domestica* fruit

Bioactive constituents	Ethanol extract
Alkaloids	-
Carbohydrate and glycosides	+
Proteins and amino acids	+
Sterols	+
Fixed oils and fats	-
Tannins and phenolic compounds	+
Triterpenoids	+
Saponins	-
Gums and mucilage	-
Flavonoids	+

+: Positive, -: Negative

Literature has shown that flavonoids[20], phenolic compounds[21], tannins[22] and terpenoids[23] show most of the antibacterial activity. *Staphylococcus aureus* is specifically more susceptible to phenolic compounds[21]. Subedi *et al.*[24]. reported that a type of flavonoid, furocoumarins inhibit bacterial growth by reacting with DNA and disrupting DNA replication. The hydrophobic character of phenolic compounds potentially impairs the cellular function and membrane integrity[25]. Tannins are responsible for the inactivation of adhesions, enzymes and cell-enveloped proteins of microbes, in conjunction with the ability to bind to the extracellular and soluble proteins[26]. Anticariogenic activity of nine labdane type-diterpenes and four sesquiterpenes against the microorganisms responsible for dental caries was reported by Souza *et al.*[27]. The presence of hydroxyl group in sesquiterpenes, which is an efficient uncoupler of the bacterial plasma membrane creates instability and breaks the membrane's phospholipid-sterol interactions and is often lethal to microorganisms[28]. All these observations explain the growth inhibitory activity of extracts of *M. domestica* fruits and provide good evidence that the fruit of *M. domestica* has antibacterial effect against the bacterial isolates tested in this study. The results of the present study corroborate the common belief that the fruits of *M. domestica* are efficacious against dental infections and would justify its further investigation to isolate potential bioactive herbal metabolites for the treatment of tooth decay.

CONCLUSION

This is a preliminary study to evaluate the antibacterial activity of *M. domestica* fruits. The crude extracts demonstrating antibacterial activity against the isolated pathogens from dental caries could result in the discovery of new chemical classes of antibiotics. Before declaring *M. domestica* fruit as a potent antimicrobial drug, further research is required on isolation of specific bioactive constituents responsible for antibacterial activity and testing the efficacy at various doses which is under process of our investigation. The study emphasizes the accuracy and efficacy of traditional remedies and inspires people to realize the importance of natural resources for their potent medicinal values.

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