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Screening of antibacterial activity of some underutilized fruits of *Sapotaceae*

[1*]Patel, P. R. and [2]Rao, T. V. R.

[1]*Agharkar Research Institute, G. G. Agarkar Road, Pune, Maharashtra – 411 004, India*
[2]*Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat – 388 120, India*

Abstract: Screening of underutilized fruits of *Sapotaceae* family *Manilkara hexandra* and *Mimusops elengi* were examined for antibacterial activity at their successive stages of growth and ripening. Zone of inhibition and minimum inhibitory concentration (MIC) were determined against medically important bacterial strains namely *Bacillus cereus, Bacillus subtilis, Micrococcus luteus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Salmonella paratyphi* and *Salmonella typhi.* The infusion extracts were used and the zone of inhibition was determined by agar well diffusion method. The methanol extracts of both the fruits screened for the present study proved to be more potent than the other extracts used. The pre-ripened and ripened stages of both the fruits exhibited moderate to high activity against most of the tested organisms. Higher activity was recorded using the fruit extracts of *Manilkara hexandra* while *Mimusops elengi* exhibited less activity against most of the tested bacterial strains. MIC value of 1 mg/ml was found effective while using methanol extract of ripened fruit of *Mimusops elengi* against *Salmonella typhi* and methanol extract of ripened fruit of *Manilkara hexandra* at 2 mg/ml inhibited *Salmonella paratyphi* and *Salmonella typhi*. Hence, the present study concludes that the underutilized fruits of *Manilkara hexandra* and *Mimusops elengi* can be further studied to discover the bioactive natural compounds that may serve and facilitate pharmacological studies leading to synthesis of a more potent drug with reduced toxicity.

Keywords: Antibacterial, growth and ripening, MIC, *Sapotaceae*, underutilized fruits

Introduction

Nature has been a source of medicinal agents since times immemorial. The importance of plant derived products in the management of human ailments cannot be overemphasized. It is clear that the plant products harbor an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Antibiotic resistance has become a global concern (Westh *et al.,* 2004). Increasing incidence of multiple resistances in bacteria in recent years, largely due to indiscriminate use of commercial antibacterial drugs commonly employed in the treatment of infectious diseases. Over the past two decades, scientists have turned back to traditional folk medicines or natural products to uncover the scientific basis of remedial effects such as antibacterial agents (Haslam, 1996).

Beside plants, fruits also have become the main subject for researchers to be investigated since their bioactive compounds related with herbs, commonly referred as phytochemicals such as carotenoids, polyphenols, anthocyanins, polyphenols such as flavonoids and tannins that are present in the fruits and vegetables such as tomatoes, grapes, pomegranates and strawberries have shown very promising results in combating various bacterial, fungal and viral diseases (Ahmad and Beg, 2001; Cushnie and Lamb, 2005; Li *et al*., 2006; Rao and Rao, 2007).

Mimusops elengi L. and *Manilkara hexandra (Roxb.) Dubard* belongs to the family Sapotaceae that includes approximately 800 species of evergreen trees and shrubs in approximately 65 genera. Many species are known to produce edible fruits and/or have other economic uses. The fruits of *Mimusops elengi* and *Manilkara hexandra* have been classified as underutilized (Peter, 2007; 2008). *Mimusops elengi* is a small evergreen tree, leaves are small shiny, thick, narrow and pointed; straight trunk and spreading branches. Flowers are small, star-shaped, yellowish white in colour while fruits are ovoid, 2.5 cm long, green in colour when raw and become yellow on ripening. Its fruits are edible and it has various traditional medicinal uses. The bark, flowers, fruits and seeds are astringent, cooling, anthelmintic, tonic and febrifuge (Patel, 2009). It is mainly used in dental ailments like chronic dysentery, constipations, bleeding gums, pyorrhea, dental caries and loose teeth. Barks are used to increase fertility in women and known to have antiulcer activity (Shah *et al.*,

*Corresponding author.
Email: *prakashpatel@inbox.com*

2003), while antifungal properties of its leaf have been reported by Satish *et al*. (2008). They are rich source of tannin, saponin, alkaloids, glucoside and ursolic acid. Seed kernel also reported to have saponins (Lavaud *et al.*, 1996).

Manilkara hexandra (Roxb.) Dubard is a large evergreen tree widely distributed throughout the greater parts of India and other tropical countries. The bark is grayish black and rough. The wood is very hard, heavy, and very durable. The stem bark is astringent, febrifuge, sweet, tonic and is used traditionally to treat a wide range of gastrointestinal symptoms (Warrier *et al*., 1995). In view of these opinions and observations, the present investigation was taken up to screen these underutilized fruits for evaluating their antibacterial activity.

Materials and Methods

The fresh underutilized fruit samples of *Manilkara hexandra (Roxb.) Dubard* and *Mimusops elengi L. (Sapotaceae)*, were collected from the vicinity of Vallabh Vidyanagar, Gujarat at their sequential stages of growth and ripening. The fruits were categorized into five sequential growth stages based on their size, shape and colour viz. (i) young (ii) pre-mature (iii) mature (iv) pre-ripened and (v) ripened stage (Patel and Rao, 2009). The fruit samples were dried at room temperature, grounded to powder and stored in air tight containers until further use. The infusion extraction method (Houghton and Raman, 1998) was used for which non polar solvent series starting from diethyl ether, ethyl acetate, acetone, methanol and water were used. The resulting extracts were concentrated by drying them at room temperature and finally stored in refrigerator until further use.

The following four gram positive and four gram negative bacterial cultures were used in this study namely *Bacillus cereus* (MTCC-430), *Bacillus subtilis* (MTCC-121), *Micrococcus luteus* (MTCC-106), *Staphylococcus epidermidis* (MTCC-435) and *Escherichia coli* (MTCC-443), *Klebsiella pneumoniae* (MTCC-109), *Salmonella paratyphi* (MTCC-735), *Salmonella typhi* (MTCC-734) respectively. All the microbial pure cultures obtained from MTCC obtained from Microbial type culture collection, Chandigarh, India (MTCC).

The antibacterial activities of these underutilized fruit extracts were screened by using agar well diffusion method (Perez *et al*., 1990). During the present study, all the used bacterial cultures were grown on nutrient agar medium (pH 7.4) at 37°C. A 0.5 Mc Farland turbidity standard was used to measure the density of bacterial cells (Ogbonnia *et al*., 2008). Antibiotics such as Ciprofloxacin and Doxycycline (20 µg/ml) were used as positive controls, while 100 and 50 % DMSO were used as negative controls. The antibacterial activities were determined by measuring the diameter of the inhibitory zone in mm. All the bioassays were carried out in triplicate to minimize the error.

The minimum inhibitory concentration (MIC) of the samples, which gave an inhibition zone of 10 mm or more, was determined. The MIC value has been evaluated using serial broth dilution method ranging from 8 mg/ml to 0.250 mg/ml. Finally the presence of live bacterial population was determined by appearance of red colour, while no colour change was observed in case of dead bacterial population, using 2, 3, 5-triphenly tetrazolium chloride test (Patel, 2009). The solutions containing DMSO and nutrient broth were used as controls. The MIC value of the samples was carried out in three replicates to confirm the activity.

Results

Mimusops elengi

Among the various extracts of *Mimusops elengi* fruit, methanol extract exhibited high inhibitory zone followed by acetone, water, ethyl acetate and diethyl ether. Diethyl ether extracts exhibited least activity (below 5 mm) against all the tested organisms. Among these bacterial strains *Staphylococcus epidermidis* and *Salmonella paratyphi* were found to be highly resistant and exhibited no activity against the *Mimusops elengi* fruit extracts. Similarly, ethyl acetate extracts of *Mimusops elengi* fruit exhibited least activity against all selected bacterial strains, among which *Bacillus cereus* and *Micrococcus luteus* were the most resistant strains, while high inhibition was recorded against *Escherichia coli*. Acetone extract of the ripened fruit exhibited good activity against *Salmonella paratyphi* (10 mm) followed by *Staphylococcus epidermidis* (12 mm) and *Micrococcus luteus* (10 mm), while mature fruit exhibited activity against *Salmonella paratyphi* (10 mm). Higher percentage of inhibition was observed using the acetone extract against *Salmonella paratyphi* followed by *Salmonella typhi,* while no activity was recorded against *Bacillus cereus*. Using the methanol extract, good activity was recorded against *Salmonella typhi* using the ripened (14 mm) and pre-ripened (11 mm) fruit extracts, besides *Escherichia coli* was found to be highly resistant when compared to that of other bacterial strains used. However, water extract of *Mimusops elengi* fruit exhibited moderate activity against *Escherichia coli* followed by *Salmonella*

Table 1. Antibacterial activity of *Mimusops elengi* fruit using infusion extracts

Infusion Extracts	Stages of fruit Development	Zone of Inhibition (mm)							
		Gram $^{+ve}$ Bacteria				Gram $^{-ve}$ Bacteria			
		BC	BS	ML	SE	EC	KP	SP	ST
Di-ethyl ether	Young	2	1	3	3	2	2	1	1
	Pre-mature	2	1	1	-	7	2	-	1
	Mature	1	1	-	2	6	-	1	1
	Pre-ripened	2	2	2	1	1	3	4	1
	Ripened	1	-	3	3	1	4	4	-
Ethyl acetate	Young	-	1	6	2	4	3	2	2
	Pre-mature	-	1	4	2	1	-	4	-
	Mature	1	1	1	8	2	2	4	-
	Pre-ripened	-	1	1	1	2	1	2	1
	Ripened	1	1	2	3	2	1	3	2
Acetone	Young	1	2	2	-	6	1	-	4
	Pre-mature	-	2	1	-	4	2	3	7
	Mature	-	2	-	7	4	1	4	2
	Pre-ripened	-	1	-	6	1	1	4	2
	Ripened	1	-	-	9	3	1	2	4
Methanol	Young	2	2	3	3	2	1	1	4
	Pre-mature	3	2	2	4	8	2	2	6
	Mature	3	2	4	4	3	2	1	9
	Pre-ripened	3	1	2	4	4	2	3	8
	Ripened	8	11	4	6	11	4	11	13
Water	Young	1	1	1	4	3	2	4	3
	Pre-mature	1	1	1	2	3	2	3	2
	Mature	1	1	2	2	3	3	3	2
	Pre-ripened	-	-	-	1	1	1	4	1
	Ripened	-	-	1	3	1	1	4	2

BC – *Bacillus cereus*, BS – *Bacillus subtilis*, EC – *Escherichia coli*, KP – *Klebsiella pneumonia*, ML – *Micrococcus luteus*, SE – *Staphylococcus epidermidis*, SP – *Salmonella paratyphi*, ST – *Salmonella typhi*

typhi, Bacillus subtilis, Salmonella paratyphi, Bacillus cereus and *Staphylococcus epidermidis*, while no activity was observed for *Micrococcus luteus* and *Klebsiella pneumoniae* (Table 1).

A minimum inhibitory concentration of 1 mg/ml from the methanol extract of the ripened fruit was helpful in controlling the growth of *Salmonella typhi.* Besides, 4 mg/ml concentration of methanol extract of pre-ripened fruit regulated the growth of *Salmonella typhi* and similar concentration of acetone extract of ripened fruit controlled the growth of bacterial strains *Staphylococcus epidermidis* and *Salmonella paratyphi* respectively. However, acetone extract of mature and ripened fruit exhibited a minimum inhibitory concentration of more than 8 mg/ml against *Salmonella paratyphi* and *Micrococcus luteus* respectively (Table 2). Mimusops elengi exhibited inhibition against three bacterial strains namely *Staphylococcus epidermidis, Salmonella paratyphi* and *Salmonella typhi*, of which *Mimusops elengi* proved to posses 100% inhibition even at 1 mg/ml against *Salmonella typhi*.

Table 2. Minimum inhibitory concentration (MIC) values of different fruit extracts.

Sr. No.	Fruit	Stage of Development	Extracts	Bacterial strains	MIC (mg/ml)
1	*Mimusops elengi*	Pre-ripened	Methanol	ST	4
		Ripened	Acetone	SE	4
				SP	4
			Methanol	ST	1
2	*Manilkara hexandra*	Ripened	Methanol	BS	4
				EC	4
				SP	2
				ST	2

BS – *Bacillus subtilis*, **EC** – *Escherichia coli*, **SE** – *Staphylococcus epidermidis*, **SP** – *Salmonella paratyphi*, **ST** – *Salmonella typhi*

Manilkara hexandra

Methanol extract of the ripened fruit of *Manilkara hexandra* exhibited good inhibition zone against *Salmonella typhi* (13 mm) followed by *Bacillus subtilis* (11 mm), *Escherichia coli* (11 mm) and *Salmonella paratyphi* (11 mm). The diethyl ether extracts of *M. hexandra* fruit demonstrated moderate zone of inhibition against *Escherichia coli* with 7 and 6 mm from pre-mature and mature fruit respectively. Least inhibitory activity was recorded against *Klebsiella pneumoniae* followed by *Salmonella paratyphi*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Bacillus subtilis* and *Salmonella typhi*. The ethyl acetate extracts of the presently studied *Manilkara hexandra* fruit exhibited moderate zone of inhibition against *Staphylococcus epidermidis* and *Micrococcus luteus* with 8 and 6 mm from the mature and young fruit respectively, while least activity was noted against *Salmonella paratyphi* (4 mm), *Escherichia coli* (4 mm), *Klebsiella pneumoniae* (3 mm), *Salmonella typhi* (1 mm), *Bacillus cereus* (1 mm) and *Bacillus subtilis* (1 mm). Acetone extracts of *Manilkara hexandra* fruit exhibited maximum zone of inhibition against *Staphylococcus epidermidis* (9 mm with ripened fruit) followed by *Salmonella typhi* (7 mm with pre-mature fruit) and *Escherichia coli* (6 mm with young fruit), while *Bacillus cereus* was found to be the most resistant bacterial strain and exhibited no inhibitory zone followed by *Micrococcus luteus, Klebsiella pneumoniae* and *Bacillus subtilis* demonstrated a 2 mm inhibitory zone, while *Salmonella paratyphi* exhibited a 4 mm inhibitory zone. Similarly, water extract exhibited least activity against the selected bacterial strains when compared to that of other extracts used. Least or no zone of inhibition was observed in *Bacillus cereus, Bacillus subtilis, Micrococcus luteus, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, Staphylococcus epidermidis* and *Salmonella paratyphi* (Table 3).

A minimum inhibitory concentration at which the growth of bacterial organisms was affected was evaluated from the fruit extracts of *Manilkara*

Table 3. Antibacterial activity of *Manilkara hexandra* fruit using infusion extracts.

Infusion Extracts	Stages of fruit Development	Zone of Inhibition (mm)							
		Gram +ve Bacteria				Gram -ve Bacteria			
		BC	BS	ML	SE	EC	KP	SP	ST
Di-ethyl ether	Young	2	1	3	3	2	2	1	1
	Pre-mature	2	1	1	-	7	2	-	1
	Mature	1	1	-	2	6	-	1	1
	Pre-ripened	2	2	2	1	1	3	4	1
	Ripened	1	-	3	3	1	4	4	-
Ethyl acetate	Young	-	1	6	2	4	3	2	2
	Pre-mature	-	1	4	2	1	-	4	-
	Mature	1	1	1	8	2	2	4	-
	Pre-ripened	-	1	1	1	2	1	2	1
	Ripened	1	1	2	3	2	1	3	2
Acetone	Young	1	2	2	-	6	1	-	4
	Pre-mature	-	2	1	-	4	2	3	7
	Mature	-	2	-	7	4	1	4	2
	Pre-ripened	-	1	-	6	1	1	4	2
	Ripened	1	-	-	9	3	1	2	4
Methanol	Young	2	2	3	3	2	1	1	4
	Pre-mature	3	2	2	4	8	2	2	6
	Mature	3	2	4	4	3	2	1	9
	Pre-ripened	3	1	2	4	4	2	3	8
	Ripened	8	11	4	6	11	4	11	13
Water	Young	1	1	1	4	3	2	4	3
	Pre-mature	1	1	1	2	3	2	3	2
	Mature	1	1	2	2	3	3	3	2
	Pre-ripened	-	-	-	1	1	1	4	1
	Ripened	-	-	1	3	1	1	4	2

BC – *Bacillus cereus*, **BS** – *Bacillus subtilis*, **EC** – *Escherichia coli*, **KP** – *Klebsiella pneumonia*, **ML** – *Micrococcus luteus*,**SE** – *Staphylococcus epidermidis* **SP** – *Salmonella paratyphi*, **ST** – *Salmonella typhi*

hexandra. A minimum inhibitory concentration value of 2 mg/ml was found to be effective against *Salmonella paratyphi* and *Salmonella typhi*, while 4 mg/ml extract was valuable to regulate the growth of *Bacillus cereus* and *Escherichia coli* (Table 2). *Manilkara hexandra* exhibited inhibition activity against four bacterial strains namely *Bacillus subtilis*, *Escherichia coli*, *Salmonella paratyphi* and *Salmonella typhi*, of which 100 % inhibition was obtained at its lowest concentration of 2 mg/ml against *Salmonella paratyphi* and *Salmonella typhi* respectively.

Discussion

The traditional medicinal methods, especially the use of plants, still play a vital role to cover the basic health needs and hence the use of herbal remedies has risen in the developed countries in the last decade (Parekh and Chanda, 2008). It is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandir *et al*., 1985).

The present study reveals that the methanol extracts of both underutilized fruits proved to be more potent than the other extracts used. The extracts of mature, pre-ripened and ripened stages of the presently studied fruits exhibited moderate to high activity against most of the organisms tested. Active compounds may be present in insufficient quantities in the crude extracts to show antibacterial activity with the dose levels employed (Taylor *et al*., 2001). Perhaps this may be the reason for not showing the MIC value in the other extracts used for this present study. Lack of activity can thus only be proven by using large doses (Farnsworth, 1993). Alternatively, if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effect or negating the positive effects of the bioactive agents (Jager *et al*., 1996). With no bacterial activity, extracts may be active against other bacterial species which were not tested (Shale *et al*., 1999).

Acetone and methanol extracts proved to posses the active antibacterial components. Pre-mature and ripened fruit of *Mimusops elengi* and ripened fruits of *Manilkara hexandra* were the optimal stages for their usage as antibacterial agents. Gram positive bacterial strains were more affected, when compared to gram negative bacterial strains. The results of the present study are in accordance with Nair and Chanda (2007); Yaghoubi *et al*. (2007), who observed gram negative bacterial strains as more resistant than that of gram positive bacterial strains.

A minimum inhibitory concentration of 1 mg/ml extract was found effective using methanol extract of ripened fruit of *Mimusops elengi* against *Salmonella typhi* and 2 mg/ml methanolic extract of *Manilkara hexandra* against *Salmonella paratyphi* and *Salmonella typhi*. Hence, ripened fruits of both fruits are highly suitable for isolation of bioactive compounds. However, the observations also demonstrate the presence of antibacterial compounds of wide specificity at different stages of maturity. Further studies are required to provide essential information about the selection of fruit extract, for isolation of bioactive constituents that are responsible for the activity. Thus the study concludes that these underutilized can be further used to find out the bioactive natural compounds that would serve and facilitate pharmacological studies leading to synthesis of a more potent drug with reduced toxicity.

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