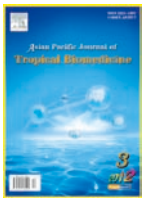
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



Document heading doi:

Evaluation of antioxidant and antimicrobial properties of *Manilkara zapota* L. (chiku) leaves by sequential soxhlet extraction method

Mital Kaneria and Sumitra Chanda*

Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University, Rajkot-360 005, Gujarat, India

ARTICLE INFO

Article history:
Received 25 August 2012
Received in revised from 5 September 2012
Accepted 7 December 2012
Available online 28 December 2012

Keywords:
Manilkara zapota
DPPH
Superoxide anion radical
Acetone extract
Total phenol content
Antimicrobial activity

ABSTRACT

Objective: Antioxidant and antimicrobial properties of *Manilkara zapota* L. (chiku) leaves was studied. **Methods:** The antioxidant property of different solvent extracts of *Manilkara zapota* L. leaves was evaluated by DPPH free radical, superoxide anion radical, hydroxyl radical scavenging activity and reducing capacity assessment, while the antimicrobial property was evaluated by agar well diffusion method against some of the tested food borne, spoilage, pathogenic and skin disease causing microorganisms. **Results:** The DPPH free radical scavenging activity of acetone extract was better than that of standard ascorbic acid and superoxide anion scavenging activity of acetone extract was better than that of standard gallic acid. It showed good reducing capacity assessment also. Maximum phenol content was also present in acetone extract thus supporting the idea that phenolic content and antioxidant activity show a direct correlation. Acetone extract showed significant antimicrobial activity amongst all the different solvent extracts. **Conclusion:** Result presented here suggest that acetone extract of *M. zapota* leaves possess strong antioxidant and antimicrobial properties, and it may be considered as an interesting and economic source of antioxidants and antimicrobics for therapeutic or nutraceutical industries and for food manufactures or pharmaceuticals.

1. Introduction

Phytochemicals are extensively found at different levels in many medicinal plants. Numerous plants used in traditional medicine are effective in treating various ailments caused by oxidative stress, bacterial and/or viral infections. Research has shown that medicinal plants exhibit antioxidant[1], as well as antimicrobial[2] activity.

Oxidative stress, which is caused mainly by free radicals, is the main cause of many pathologic conditions such as inflammation, arthritis rheumatoid, cancer, neurodegenerative disorders and ageing and antioxidant rich diets are able to prevent and reduce these abnormalities effectively[3]. Free radicals, reactive oxygen species (ROS), chemical reactions, and several redox reactions of various compounds may cause protein oxidation, DNA damage, and lipid peroxidation in living cells[4]. ROS includes superoxide radical ($O2^{-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH), are cytotoxic and give rise to tissue injuries, leading to cellular damage, metabolic disorders, and senescence processes[5]. Excessive amount of ROS is harmful because they initiate bimolecular oxidation which leads to cell death and creates oxidative stress. In addition, oxidative stress causes inadvertent enzyme activation and oxidative damage to cellular system[6].

Antioxidants are the compounds that, when added to food products, act as radical scavengers, prevent the radical chain reactions of oxidation, delay or inhibit the oxidation process and increase shelf life by retarding the process of lipid peroxidation[7]. Many biochemical and pharmacological studies have reported that polyphenols of various herbs are beneficial to human health. Some bioactive substances in polyphenol-rich plants have been made into functional foods or supplements[8].

Because of increasing resistance to antibiotics of many bacteria, plant extracts and plant compounds are of new interest as antiseptics and antimicrobial agents in medicine. The efficacy of the plants in curing various ailments is well established and a large volume of work has been done in this field by researchers in India and abroad[9-11]. The global emergence of multi-drug resistant bacterial strains is increasingly limiting the effectiveness of current drugs and significantly causes treatment failure of infections[12]. Examples include methicillin-resistant *staphylococci*, *pneumococci* resistant to penicillin and macrolides, vancomycin-resistant *enterococci* as well as multi-drug resistant Gram-negative organisms[13].

*Corresponding author: Sumitra Chanda, Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University, Rajkot-360 005, Gujarat, India
E-mail: svchanda@gmail.com
Tel. No. +91 9426247893

Manilkara zapota L. (Sapotaceae), is an evergreen, glabrous tree, 8-15 m in height, cultivated throughout India, though it is native to Mexico and Central America. Fruits are edible, sweet with rich fine flavour. The seeds are aperients, diuretic tonic and febrifuge. Bark is antibiotic, astringent and febrifuge. Chicle from bark is used in dental surgery. Bark is used as tonic and the decoction is given in diarrhoea, dysentery and peludism[14, 15]. The leaves are used to treat cough, cold, and diarrhea[14, 16]. Antimicrobial and antioxidant activities are also reported from the leaves[17, 18]. These reports suggest that *M. zapota* can probably be used as a health food.

The objective of this research work was to investigate the antioxidant and antimicrobial properties from different solvent extracts of leaves of *M. zapota* obtained by sequential extraction method by soxhlet apparatus.

2. Material and methods

2.1 Chemicals

2, 2-diphenyl-1picrylhydrazyl (DPPH), Nitroblue tetrazolium (NBT), Phenazine methosulfate (PMS), Nicotinamide Adenine Dinucleotide reduced (NADH), gallic acid, ascorbic acid, quercetin, Folin-Ciocalteu's reagent, aluminium chloride, potassium acetate, 2-deoxy-D-ribose, thiobarbituric acid, trichloroacetic acid, hydrogen peroxide, ethylenediamine tetraacetic acid (EDTA), ferric chloride, potassium ferricyanide, Tris-HCl, Dimethyl sulfoxide (DMSO), nutrient broth, Sabouraud dextrose broth, Muller Hinton No. 2, Sabouraud dextrose agar, agar powder were obtained from Hi-Media, Mumbai; petroleum ether, toluene, acetone, ethyl acetate, methanol, etc were obtained from Merck, India.

2.2 Plant material

Fresh leaves of *Manilkara zapota* L. were collected in the month of August, 2008, from Jam-jodhpur, Jamnagar, Gujarat, India. The plant was compared with voucher specimen (voucher specimen No. PSN429) deposited at Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The leaves were separated, washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in air tight bottles.

2.3 Extraction method

The dried powder of the leaves was extracted sequentially[19] by soxhlet apparatus[20], using different solvents depending upon their polarities like petroleum ether, toluene, ethyl acetate, acetone and water (Flow chart 1). The extracts were concentrated and freed of solvent under reduced pressure, using rotary evaporator. The dried crude concentrated extracts were weighed to calculate the extractive yield and stored in a refrigerator (4℃) in air tight bottles, until used for analysis.

2.4 Quantitative phytochemical analysis

2.4.1 Determination of total phenol content

The amount of total phenol content, in different solvent extracts of leaves was determined by Folin-Ciocalteu's reagent method[21]. 0.5 ml of extract and 0.1 ml (0.5 N) Folin-Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate solution was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm. Gallic acid was used as a positive control. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of extracted compounds).The assay was carried out in triplicate and the mean values with ±SEM is presented.

2.4.2 Determination of total flavonoid content

The amount of flavonoid content in different solvent extracts of leaves was determination by aluminium chloride colorimetric method[22]. The reaction mixture 3 ml consisted of 1 ml of sample (1 mg/ml) and 0.5 ml of (1.2%) aluminium chloride and 0.5 ml (120 mM) potassium acetate was incubated at room temperature for 30 min. The absorbance of all samples was measured at 415 nm. Quercetin was used as positive control. The flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compound). The assay was carried out in triplicate and the mean values with ±SEM are presented.

2.5 Antioxidant Assays

2.5.1 DPPH free radical scavenging activity

The free radical scavenging activity of different solvent extracts of leaves was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) by the modified method of Mc Cune and Johns[23]. The reaction mixture 3.0 ml consisted of 1.0 ml of DPPH (0.3 mM), 1.0 ml of extract (different concentrations) and 1.0 ml of methanol, was incubated for 10 min, in dark, after which the absorbance was measured at 517 nm. Ascorbic acid was used as positive control[24]. The assay was carried out in triplicate and the mean values with±SEM are presented. The percentage inhibition was determined by comparing the results of the test and the control. Percentage of inhibition was calculated using the formula

$$\% \text{ Inhibition} = [1 - (A/B)] \times 100$$

Where, B = absorbance of blank (DPPH, plus methanol)
A = the absorbance of sample (DPPH, methanol plus sample)

2.5.2 Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of different solvent extracts of leaves was measured by studying the competition between deoxyribose and test compound for hydroxyl radicals generated by Fe^{+3}-Ascorbic acid-EDTA-H_2O_2 system (Fenton reaction) according to the method of Kunchandy and Rao[25]. The reaction mixture 1.0 ml consisted of 100 μl of 2-deoxy-D-ribose (28 mM in 20 mM KH^2PO^4-KOH buffer, pH 7.4), 500 μl of the various solvent extracts, 200 μl EDTA (1.04 mM) and 200 μM $FeCl_3$ (1:1 v/v), 100 μl 1.0 mM H_2O_2 and 100 μl ascorbic acid (1.0 mM), was incubated at 37℃ for 1 h. 1.0 ml of thiobarbituric acid

(1%) and 1 ml of trichloroacetic acid (2.8%) was added and incubated at 100℃ for 20 min. After cooling, the absorbance of pink colour was measured at 532 nm against a blank sample. Gallic acid was used as a positive control[25]. The assay was carried out in triplicate and the mean values with±SEM are presented. The percentage inhibition was determined by comparing the results of the test and the control.

2.5.3 Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity of different solvent extracts of leaves was measured by the method as described by Robak and Gryglewski[26]. Superoxide radicals are generated by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). The reaction mixture 3 ml consisted of 0.5 ml of NBT (0.3 mM), 0.5 ml of Tris-HCl buffer (16 mM, pH 8), 0.5 ml NADH (0.936 mM), 0.5 ml PMS (0.12 mM) and 1 ml of different concentrations of different solvent extracts. The superoxide radical generating reaction was started by the addition of 0.5 ml of phenazine methosulfate (PMS) solution to the mixture. The reaction mixture was incubated at 25℃ for 5 min and then the absorbance was measured at 560 nm against a blank sample. Gallic acid was used as a positive control[26]. The assay was carried out in triplicate and the mean values with±SEM are presented. The percentage inhibition was determined by comparing the results of the test and the control.

2.5.4 Reducing capacity assessment

The reducing capacity assessment of different solvent extracts of leaves was determined using method as described by Athukorala et al.[27]. 1.0 ml of different concentrations of solvent extracts was mixed with 2.5 ml of potassium phosphate buffer (200 mM, pH 6.6) and potassium ferricyanide (2.5 ml, 30 mM). The mixture was then incubated at 50℃ for 20 min. There after 2.5 ml of trichloroacetic acid (600 mM) was added to the reaction mixture and then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of $FeCl_3$ (6 mM) and the absorbance was measured at 700 nm. Ascorbic acid was used as positive control[28]. The assay was carried out in triplicate and the mean values with±SEM are presented.

2.6 Antimicrobial assay

2.6.1 Microorganisms tested

The bacterial and fungal strains used to assess the antibacterial properties of different solvent extracts of *M. zapota* included five Gram positive bacteria (*Bacillus megaterium* ATCC9885, *Bacillus subtilis* ATCC6633, *Corynebacterium rubrum* ATCC14898, *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* ATCC12228), five Gram negative bacteria (*Citrobacter freundii* ATCC10787, *Enterobacter aerogenes* ATCC13048, *Klebsiella pneumoniae* NCIM2719, *Proteus mirabilis* NCIM2241, *Salmonella typhimurium* ATCC23564) and five fungi (*Candida albicans* ATCC2091, *Candida glabrata* NCIM3448, *Cryptococcus leuteolus* ATCC32044, *Candida neoformans* NCIM3542, *Candida tropicalis* ATCC4563). The investigated bacterial and fungal strains were obtained from National Chemical Laboratory (NCL), Pune, India. The organisms were maintained on nutrient agar and sabouraud dextrose agar (Hi Media, India) respectively bacteria and fungi, slop at 4℃ and sub-cultured before use. The microorganisms studied are clinically important ones causing several infections, food born diseases, spoilages, skin infection and it is essential to overcome them through some active therapeutic agents.

2.6.2 Determination of antimicrobial assay

In vitro antimicrobial activity of the different solvent extracts of *M. zapota* was studied against fifteen microbial strains by the agar well diffusion method[29, 30]. Muller Hinton No. 2 / Sabouraud dextrose agar (Hi-media) was used for the antibacterial and antifungal susceptibility test respectively. The extracts were diluted in 100% dimethylsulphoxide (DMSO) at the concentration of 20 mg/ml. The microbial activity was evaluated at the concentration 2.0 mg/well. The Muller Hinton agar / Sabouraud dextrose agar was melted and cooled to 48-50℃ and a standardized inoculum (1.5×10^8 CFU/ml, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile Petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 μl) was introduced in the well (8.5 mm). The plates were incubated over night at 37℃ for 24 h and 28℃ for 48 h of bacteria and fungi respectively. The antimicrobial spectrum of the extract was determined for the bacterial and fungal species in terms of zone sizes around each well. DMSO was used as negative control. The control zones were subtracted from the test zones and the resulting zone diameter is shown in the Table 7. The experiment was performed three times to minimize the error and the mean values±SEM are presented.

3. Results

3.1 Extractive yield

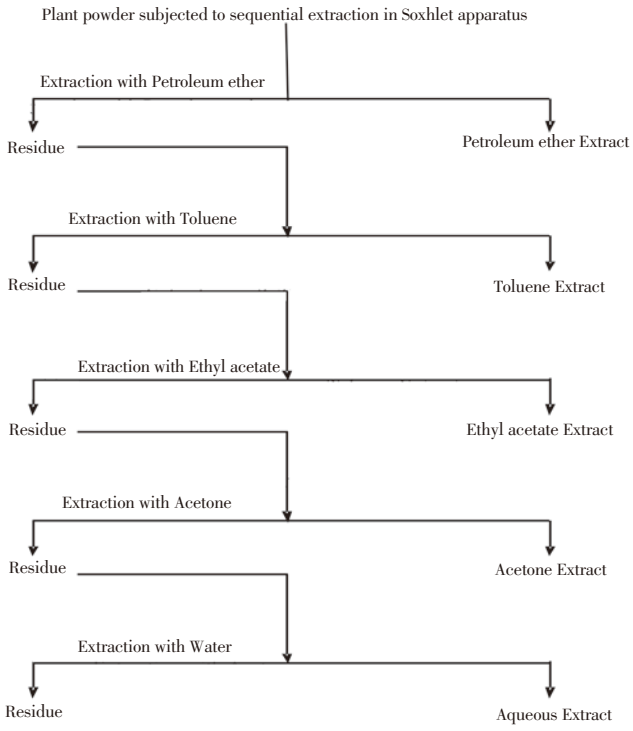
The extractive yield of different solvent extracts of *M. zapota* leaves is given in table 1. The extractive yield varied among different solvents used. Aqueous extract showed highest extractive yield than the other extracts. The extractive yield can be ranked from high to low in the following order aqueous extract (16.26) > petroleum ether extract (7.32) > acetone extract (2.98) > toluene extract (1.55) > ethyl acetate extract (0.71).

Table 1

Extractive yield, total phenol and flavonoid content of different solvent extracts of leaves of *Manilkara zapota*

Different solvent extract	% Yield (w/w)	Total phenol content (mg/g)	Flavonoid content (mg/g)
Petroleum ether	7.32±0.40	ND	ND
Toluene	1.55±0.09	4.45±0.09	59.84±0.59
Ethyl acetate	0.71±0.04	137.63±1.12	127.63±0.20
Acetone	2.98±0.02	241.06±0.81	166.84±0.31
Aqueous	16.26±0.60	106.19±1.99	37.04±0.37

ND: Not done; Values are expressed in mean±SEM (n = 3)



Flow Chart 1. Systematic representation of preparation of different solvent extracts of leaves of *Manilkara zapota* by sequential extraction method.

3.2 Total phenol and flavonoid contents

In the present work, total phenolic content was more than the flavonoid content except in toluene extract (Table 1). The ethyl acetate extract had almost same amount of total phenol and flavonoid content. Highest total phenolic and flavonoid content was present in acetone extract followed by ethyl acetate extract (Table 1).

3.3 Antioxidant activities

3.3.1 DPPH free radical scavenging activity

Out of four extracts investigated, only toluene extract showed IC50 value more than 1000 μg/ml (Table 2), the remaining three extracts showed a varied level of DPPH free radical scavenging activity (Table 3). IC_{50} values ranged from 7.6 μg/ml to 42.5 μg/ml (Table 2). The IC_{50} value of acetone extract was 7.6 μg/ml, which was better then that of standard ascorbic acid 11.4 μg/ml (Table 2). The IC_{50} value of ethyl acetate extract was 22 μg/ml while that of aqueous extract was 42.5 μg/ml (Table 2).

3.3.2 Hydroxyl radical scavenging activity

Out of four extracts investigated, toluene and acetone extracts showed IC50 value more than 1000 μg/ml (Table 2), the remaining two extracts showed a varied level of hydroxyl radical scavenging activity (Table 4). The IC_{50} value of ethyl acetate extract was 540 μg/ml while that of aqueous extract was 700 μg/ml (Table 2). The ethyl acetate extract appears to be a better scavenger of hydroxyl radical than aqueous extract.

3.3.3 Superoxide anion radical scavenging activity

Out of four extracts investigated, only toluene extract showed IC_{50} value more than 1000 μg/ml (Table 2), the remaining three extracts showed a varied level of superoxide anion radical scavenging activity (Table 5). IC_{50} values ranged from 78 μg/ml to 385 μg/ml (Table 2). The IC_{50} value of aqueous extract was 270 μg/ml while that of ethyl acetate extract was 385 μg/ml (Table 2). The IC_{50} value of acetone extract was 78 μg/ml, which was better than that of standard gallic acid (185 μg/ml) (Table 2).

3.3.4 Reducing capacity assessment

In *M. zapota* leaves, there was concentration dependent

Table 2.

IC_{50} Values of DPPH free radical scavenging activity (DPPH), Hydroxyl radical scavenging activity (OH) and Superoxide anion radical scavenging activity (SO) of different solvent extracts of leaves of *Manilkara zapota*

Antioxidant assays	IC_{50} Values (μg/ml)					
	Standard		Extracts			
	Gallic acid	Ascorbic acid	Toluene	Ethyl acetate	Acetone	Aqueous
DPPH	ND	11.4	–	22	7.6	42.5
OH	140	ND	–	540	–	700
SO	185	ND	–	385	78	270

–: >1000 μg/ml; ND = Not Done

Table 3.

DPPH free radical scavenging activity of different solvent extracts of leaves of *Manilkara zapota*

Standard Ascorbic acid		Ethyl acetate extract		Acetone extract		Aqueous extract	
Conc. (μg/ml)	% Inhibition	Conc. (μg/ml)	% Inhibition	Conc. (μg/ml)	% Inhibition	Conc. (μg/ml)	% Inhibition
2	8.60	5	11.62±0.010	2	11.04±0.001	10	9.86±0.007
4	16.60	10	21.35±0.009	4	25.65±0.008	20	25.28±0.010
8	31.33	15	31.53±0.003	6	39.60±0.006	30	35.62±0.004
12	48.65	20	47.56±0.041	8	52.80±0.009	40	46.33±0.012
14	59.70	25	56.48±0.001	10	65.26±0.007	50	56.67±0.004
16	69.79	30	66.75±0.007	12	75.46±0.002	60	65.41±0.002

Values are expressed in mean±SEM (n = 3)

Table 4.

Hydroxyl radical scavenging activity of different solvent extracts of leaves of *Manilkara zapota*

Standard Gallic acid		Ethyl acetate extract		Aqueous extract	
Concentration (μg/ml)	% Inhibition	Concentration (μg/ml)	% Inhibition	Concentration (μg/ml)	% Inhibition
20	8.67±1.469	200	41.70±0.009	200	16.73±0.000
60	23.31±0.872	400	49.07±0.015	400	29.75±0.000
100	29.54±0.419	600	56.25±0.004	600	43.70±0.001
140	49.96±1.328	800	70.32±0.001	800	56.46±0.001
160	75.38±0.395	1000	75.65±0.001	1000	65.93±0.000

Values are expressed in mean±SEM (n = 3)

Table 5.

Superoxide anion radical scavenging activity (SO) of different solvent extracts of leaves of *Manilkara zapota*

StandardGallic acid		Ethyl acetate extract		Acetone extract		Aqueous extract	
Conc. (μg/ml)	% Inhibition	Conc. (μg/ml)	% Inhibition	Conc. (μg/ml)	% Inhibition	Conc. (μg/ml)	% Inhibition
50	11.88±1.459	100	25.96±0.002	20	11.66±0.002	100	20.77±0.004
100	24.94±0.355	200	38.05±0.003	40	24.15±0.006	200	38.48±0.007
150	41.37±0.546	300	44.79±0.001	60	39.20±0.014	300	58.64±0.008
200	52.17±0.055	400	51.83±0.003	80	52.61±0.001	400	71.23±0.006
225	66.66±0.000	500	63.62±0.001	100	64.79±0.003	500	79.73±0.005

Values are expressed in mean±SEM (n = 3)

Table 6.

Reducing capacity assessment of different solvent extracts of leaves of *Manilkara zapota*

Standard Ascorbic acid		Toluene extract		Ethyl acetate extract		Acetone extract		Aqueous extract	
Conc. (μg/ml)	Reducing power (Absorbance at 700 nm)	Conc. (μg/ml)	Reducing power (Absorbance at 700 nm)	Conc. (μg/ml)	Reducing power (Absorbance at 700 nm)	Conc. (μg/ml)	Reducing power (Absorbance at 700 nm)	Conc. (μg/ml)	Reducing power (Absorbance at 700 nm)
20	0.186±0.000	20	0.002±0.000	20	0.060±0.003	20	0.152±0.001	20	0.066±0.001
40	0.365±0.001	40	0.008±0.001	40	0.161±0.000	40	0.291±0.000	40	0.147±0.000
60	0.542±0.022	60	0.007±0.000	60	0.197±0.001	60	0.389±0.000	60	0.213±0.004
80	0.670±0.005	80	0.010±0.000	80	0.238±0.001	80	0.492±0.000	80	0.250±0.000
100	0.762±0.020	100	0.012±0.000	100	0.281±0.001	100	0.585±0.002	100	0.289±0.000
120	0.897±0.007	120	0.014±0.000	120	0.318±0.001	120	0.712±0.001	120	0.336±0.002
140	1.083±0.031	140	0.017±0.000	140	0.355±0.000	140	0.879±0.000	140	0.379±0.001
160	1.298±0.001	160	0.017±0.000	160	0.398±0.001	160	0.891±0.001	160	0.427±0.001
180	1.429±0.006	180	0.023±0.001	180	0.444±0.000	180	0.950±0.005	180	0.463±0.001

Values are expressed in mean±SEM (n = 3)

increase in the absorbance of reaction mixture for all the three extracts and standard, ascorbic acid (Table 6). Out of four studied extracts, toluene extract showed poor reducing capacity. The acetone extract showed maximum absorbance and hence maximum reducing capacity assessment among its various solvent extracts (Table 6). The reducing capacity assessments of extracts were in the order: ascorbic acid > acetone extract > aqueous extract > ethyl acetate extract > toluene extract.

3.4 Antimicrobial activity

The results of antimicrobial activity are shown in Table 7. The acetone and aqueous extracts showed better activity amongst all the extracts studied. Highest inhibition zone was shown by acetone extract against *K. pneumoniae*. The acetone extract showed activity against all the Gram positive and Gram negative bacteria except *C. freundii* and *E. aerogenes*.

4. Discussion

Extraction is critical to the recovery of antioxidant phytochemicals; under the same time and temperature conditions, the solvents used and the chemical property of samples are the two most important factors[31]. There are many reports in the literature where extractive yield varied with different solvents[18, 32, 33]. Polyphenols are widely distributed in the plant kingdom have been used to treat many human diseases. The biological activities are believed to be due to their redox properties which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[34].

Antioxidant activity is a dependent system and the characteristic of a particular system can influence the outcome of the analysis. Hence, a single assay would not be representative of the antioxidant potential of plant

Table 7.

Antimicrobial activity of different solvent extracts of leaves of *Manilkara zapota*

Micro-organisms		Zone of Inhibition (mm)				
		Petroleum ether	Toluene	Ethyl acetate	Acetone	Aqueous
Gram positive	BM	–	–	–	10.5±0.29	–
	BS	–	–	–	9.0±0.00	–
	CR	–	–	–	12.5±0.29	9.5±0.29
	SA	–	–	–	14.0±0.00	10.5±0.29
	SE	–	–	–	12.5±0.29	11.0±0.00
Gram negative	CF	–	–	–	–	–
	EA	–	–	–	–	–
	KP	11.0±0.29	–	–	15±0.29	11.5±0.00
	PM	–	–	–	13.5±0.29	10.0±0.00
	ST	–	–	–	10.5±0.29	10.0±0.00
Fungi	CA	–	–	–	–	9.5±0.29
	CG	9±0.00	–	–	–	10.0±0.00
	CL	–	–	–	–	–
	CN	9±0.00	10.5±0.29	–	–	–
	CT	–	–	–	–	–

–: No inhibition; BM: *Bacillus megaterium*; BS: *Bacillus subtilis*; CR: *Corynebacterium rubrum*; SA: *Staphylococcus aureus*; SE: *Staphylococcus epidermidis*; CF: *Citrobacter freundii*; EA: *Enterobacter aerogenes*; KP: *Klebsiella pneumoniae*; PM: *Proteus mirabilis*; ST: *Salmonella typhimurium*; CA: *Candida albicans*; CG: *Candida glabrata*; CL: *Cryptococcus leuteolus*; CN: *Candida neoformans*; CT: *Candida tropicalis*; Values are presented in mean±SEM (n=3)

extracts[35]. Therefore, in the present study, the antioxidant capacity of *M. zapota* leaves, against ROS species was accessed by four different *in vitro* assays. The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form diphenypicrylhydrazine (non radical) with the loss of this violet colour[36]. Low IC_{50} value indicates high antioxidant activity. Therefore it can be stated that acetone extract possess strong antioxidant activity. Silva et al.[37] reported the significant scavenging effects of phenolic compounds against the DPPH free radical. It is generally believed that plants which are having more phenolic content show good antioxidant activity that is there is a direct correlation between total phenol content and antioxidant activity[38, 39]. Phenolic compounds of the acetone extract of *M. zapota* were probably involved in their antiradical activity. Kaneria et al.[18] showed that methanolic extract of *M. zapota* leaves rich in phenolic content showed good DPPH free radical scavenging activity. Hydroxyl radicals are known to be the most reactive of all the reduced forms of dioxygen, and are capable of damaging almost every molecule found in living cells[40]. These radicals have the capacity to join the nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity[41]. This antioxidant assay showed weak correlation with phenolic content. The acetone extract had more phenolic content but less hydroxyl radical scavenging activity while ethyl acetate extract had low amount of phenol content but showed more hydroxyl radical scavenging. This suggests that non-phenolic compounds may also be responsible for the observed antiradical activity as also suggested by Yam et al.[42]. The superoxide anion is the most common free radical generated *in vivo*. Under oxidative stress, the concentration of this species can increase dramatically in all cells, inducing several pathophysiological processes, due to its transformation into more reactive species[43]. Above data suggests that acetone extract of *M. zapota* is strong superoxide anion quencher; the constituents of acetone extract of *M. zapota* leaves is capable of scavenging reactive species such as superoxide via a mechanism of electron/ hydrogen donation and should be able to prevent oxidative damage of the major bio-molecules: proteins and lipids as also suggested by Kaur et al.[44]. Various mechanisms, including reducing capacity, prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging have been claimed to explain the antioxidant activities[45]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[46, 47]. These results again show a good relationship between total phenols and antioxidant activity. Overall, the antioxidant activities of the extracts were highly correlated with their total phenolic contents and these results are similar to those of previous findings[48-52].

The medicinal plants play a vital role in covering the basic health needs and offer a new source of antibacterial and antifungal agents with significant activity against pathogenic microorganisms[53]. In the present work, the plant extracts showed more antibacterial activity than antifungal activity; Gram-positive bacteria were more susceptible than Gram-negative bacteria. This is similar to the general belief that Gram-positive bacteria are more susceptible to herbal drugs than Gram-negative bacteria[54-56].

In this work, the acetone extract showed good antioxidant activity in three different assays and also showed a broad spectrum of antibacterial activity against Gram positive and Gram negative bacteria. These data suggest potential effects of sequence of solvents of different polarity on the antioxidant and antimicrobial properties. Therefore, it is

essential to use more than one solvent before assessing the antioxidant property of any plant.

5. Conclusion

According to the results, acetone extract of *M. zapota* has significant antioxidant activity as well as remarkable antimicrobial activity indicating a possible preventive role in several disorders. Moreover, *M. zapota* can be used as an easily accessible and rich source of natural antioxidants and antimicrobics, as a food supplement or in the pharmaceutical industry. The various antioxidant mechanisms of acetone extract of *M. zapota* may be attributed to its strong abilities as a hydrogen donor and remarkable scavenger of ROS. In addition, the antioxidant activity may be due to phenolic compounds in acetone extract of *M. zapota*. However, the components responsible for the activities of acetone extract of *M. zapota* are currently unclear. Therefore, it is suggested that further work be performed on the isolation and identification of the bioactive components, which may result in a modern drug from this plant. Work in this direction is in progress.

Acknowledgment

The authors thank Prof. S.P. Singh, Head, Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India for providing excellent research facilities. One of the author Mr. Mital Kaneria is thankful to University Grants Commission, New Delhi, India for providing financial support as Junior Research Fellowship.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Kiselova Y, Ivanova D, Chervenkov T, Gerova D, Galunska B, Yankova T. Correlation between the *in vitro* antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. *Phytother Res* 2006; **20**: 961-965.

[2] Chan LW, Cheah ELC, Saw CLL, Weng W, Heng PWS. Antimicrobial and antioxidant activities of Cortex Magnoliae Officianalis and some other medicinal plants commonly used in South-East Asia. *Chinese Med* 2008; **3**: 15.

[3] Feillet-Coudray C, Sutra T, Fouret G, Ramos J, Wrutniak-Cabello C, Cabello G, et al. Oxidative stress in rats fed a high-fat high sucrose diet and preventive effect of polyphenols: involvement of mitochondrial and NAD(P)H oxidase systems. *Free Rad Biol Med* 2009; **46**: 624-632.

[4] Kalaivani T, Mathew I. Free radical scavenging activity from leaves of *Acacia nilotica* (L.) Wild. ex Delile, an indian medicinal tree. *Food Chem Toxicol* 2010; **48**: 298-305.

[5] Jainu M, Devi CSS. *In vitro* and *in vivo* evaluation of free radical scavenging potential of *Cissus quadrangularis*. *Afr J Biomed Res* 2005; **8**: 95-99.

[6] Prasad NK, Xie H, Hao J, Yang B, Qiu S, Wei X, et al. Antioxidant and anticancer activities of 8-hydroxypsoralen isolated from wampee [Clausena lansium (Lour.) *Skeels] peel*. *Food Chem* 2010; **118**: 62-66.

[7] Souza JNS, Silva EM, Loir A, Rees JF, Rogez H, Larondelle Y. Antioxidant capacity of four polyphenol-rich Amazonian plant extracts: a correlation study using chemical and biological in vitro assays. *Food Chem* 2008; **106**: 331-339.

[8] Kim TJ, Weng WL, Stojanovic J, Lu Y, Jung YS, Silva JL. Antimicrobial effect of water-soluble muscadine seed extracts on Escherichia coli O157:H7. *J Food Protect* 2008; **71**: 1465-1468.

[9] Stojkovic D, Sokovic M, Glamoclija J, Dzamic A, Ciric A, Ristic M, Grubišic D. Chemical composition and antimicrobial activity of Vitex agnus-castus L. fruits and leaves essential oils. *Food Chem* 2011; **128**: 1017-1022.

[10] Tyagi AK, Malik A. Antimicrobial potential and chemical composition of Eucalyptus globulus oil in liquid and vapour phase against food spoilage microorganisms. *Food Chem* 2011; **126**: 228-235.

[11] Chanda S, Kaneria M, Baravalia Y. Antioxidant and antimicrobial properties of various polar solvent extracts of stem and leaves of four Cassia species. *Afr J Biotechnol* 2012; **11**: 2490-2503.

[12] Hancock EW. Mechanisms of action of newer antibiotics for Gram positive pathogens. *Lancet Infect Dis* 2005; **5**: 209-218.

[13] Norrby RS, Nord CE, Finch R. Lack of development of new antimicrobial drugs; a potential serious threat to public health. Lancet *Infect Dis* 2005; **5**: 115-119.

[14] Mohiddin HMYB, Chin W, Holdsworth D. Traditional medicinal plants of Brunei, Darussalam Part III. Sengkurong. *Int J Pharmacog* 1992; **30**: 105-108.

[15] Anjaria J, Parabia M, Dwivedi S. Ethnovete heritage Indian Ethnoveterinary Medicine, an overview, Pathik enterprise, Ahmedabad, India, 2002.

[16] Morton JF. Fruits of Warm Climates. *Creative resources systems Inc*. 1987; 393-398.

[17] Nair R, Chanda S. Antimicrobial activity of Terminalia catappa, *Manilkara zapota* and Piper betel leaf extract. *Indian J Pharma Sci* 2008; **70**: 390-393.

[18] Kaneria M, Baravalia Y, Vaghasiya Y, Chanda S. Determination of antibacterial and antioxidant potential of some medicinal plants from Saurashtra region, India. *Indian J Pharma Sci* 2009; **71**: 406-412.

[19] Wiart C, Hannah A, Yassim M, Hamimah H, Sulaiman M. Antimicrobial activity of *Acalyoha siamensis* Oliv. Ex Gage. *J Ethnopharmacol* 2004; **95**: 285-286.

[20] Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jager AK, et al. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and antimicrobial activities. *J Ethnopharmacol* 1999; **68**: 267-274.

[21] Mc Donald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. *Food Chem* 2001; **73**: 73-84.

[22] Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in *Propolis* by two complementary colorimetric methods. *J Food Drug Anal* 2002; **10**: 178-182.

[23] Mc Cune LM, Johns T. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by

the indigenous peoples of the North American boreal forest. *J Ethnopharmacol* 2002; **82**: 197-205.

[24]Liu J, Wang C, Wang Z, Zhang C, Lu S, Liu J (2011). The antioxidant and free-radical scavenging activities of extract and fractions from corn silk (*Zea mays* L.) and related flavone glycosides. *Food Chem* 2011; **126**: 261-269.

[25]Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcumin. *Int J Pharma* 1990; 58: 237-240.

[26]Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. *Biochem Pharmacol* 1988; **37**: 837-841.

[27]Athukorala Y, Kim KN, Jeon YJ. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga *Ecklonia cava*. *Food Chem Toxicol* 2006; **44**: 1065-1074.

[28]Kumar MSY, Dutta R, Prasad D, Misra K. Subcritical water extraction of antioxidant compounds from Sea buckthorn (Hippophae rhamnoides) leaves for the comparative evaluation of antioxidant activity. *Food Chem* 2011; **127**: 1309-1316.

[29]Perez C, Paul M, Bazerque P. An antibiotic assay by the agar well diffusion method. *Acta Bio Med Exp* 1990; **15**: 113-115.

[30]Chanda S, Kaneria M. Indian nutraceutical plant leaves as a potential source of natural antimicrobial agents. In A. Mendez-Vilas (Ed.), Science against Microbial Pathogens: Communicating Current Research and Technological Advances, Formatex Research Center, *Spain* 2011; **2**: 1251-1259.

[31]Shimada K, Fujikawa K, Yahara R, Nakamura T. Antioxidative properties of xanthan on autoxidation of soybean oil in cyclodextrin emulsion. *J Agric Food Chem* 1992; **40**: 945-948.

[32]Yang D, Wang Q, Ke L, Jiang J, Ying T. Antioxidant activities of various extracts of lotus (*Nelumbo nuficera* Gaertn) rhizome. *Asia Pac J Clin Nutr* 2007; **16**: 158-163.

[33]Kaneria MJ, Bapodara MB, Chanda SV. Effect of extraction techniques and solvents on antioxidant activity of Pomegranate (*Punica granatum* L.) leaf and stem. *Food Anal Methods* 2011; Doi: 10.1007/s12161-011-9257-6.

[34]Itagaki S, Kurokawa T, Nakata C, Saito Y, Oikawa S, Kobayashi T, et al. *In vitro* and *in vivo* antioxidant properties of ferulic acid: a comparative study with other natural oxidation inhibitors. *Food Chem* 2009; **114**: 466-471.

[35]Chanda S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *Afr J Microbiol Res* 2009; **3**: 981-996.

[36]Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J Sci Technol* 2004; 26: 211-219.

[37]Silva FAM, Boryer F, Guimaraes C, Lima JLFC, Matos C, Reis C. Phenolic acids and derivatives: studies on the relationship among structure, radical scavenging activity and physicochemical parameters. *J Agric Food Chem* 2000; **48**: 21-22.

[38]Baravalia Y, Kaneria M, Vaghasiya Y, Parekh J, Chanda S. Antioxidant and antibacterial activity of *Diospyros ebenum* Roxb. leaf extracts. *Turk J Biol* 2009; **33**: 159-164.

[39]Gholivand MB, Nasrabadi MR, Batooli H, Ebrahimabadi AH. Chemical composition and antioxidant activities of essential oil and methanol extracts of *Psammogeon canescens*. *Food Chem Toxicol* 2010; **48**: 24-28.

[40]Lee JC, Kim HR, Kim J, Jang YS. Antioxidant property of an ethanol extract of the stem of Opuntia ficus-indica var. Saboten. *J Agric Food Chem* 2002; **50**: 6490-6496.

[41]Moskovitz J, Yim KA, Choke PB. Free radicals and disease. *Arch Biochem Biophys* 2002; **397**: 354-359.

[42]Yam MF, Basir R, Asmawi MZ, Ahmad MR, Akowuah GA. Antioxidant and hepatoprotective activities of *Elephantopus tomentosus* ethanol extract. *Pharma Biol* 2008; **46**: 199-206.

[43]Gulcin I, Elias R, Gepdiremen A, Boyer L, Koksal E. A comparative study on the antioxidant activity of fringe tree (*Chionanthus virginicus* L.) extracts. *Afr J Biotechnol* 2007; **6**: 410-418.

[44]Kaur A, Pan M, Meislin M, Facciotti MT, El-Geweley R, Baliga NS. A systems view of haloarchaeal strategies to withstand stress from transition metals. *Genomic Res* 2006; **16**: 841-854.

[45]Yildirim A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *J Agric Food Chem* 2001; **49**: 4083-4089.

[46]Chanda S, Dave R, Kaneria M. In vitro antioxidant property of some Indian medicinal plants. *Res J Med Plant* 2011; **5**: 169-179.

[47]Chanda SV, Kaneria MJ. Optimization of conditions for the extraction of antioxidants from leaves of *Syzygium cumini* L. using different solvents. *Food Anal Methods* 2011; Doi:10.1007/s12161-011-9242-0.

[48]Park EJ, Jhon DY. The antioxidant, angiotensin converting enzyme inhibition activity, and phenolic compounds of bamboo shoot extracts. *LWT - Food Sci Technol* 2010; **43**: 655-659.

[49]Lee OH, Lee BY. Antioxidant and antimicrobial activities of individual and combined phenolics in *Olea europaea* leaf extract. *Bioresource Technol* 2010; **101**: 3751-3754.

[50]Perez MB, Banek SA, Croci CA. Retention of antioxidant activity in gamma irradiated argentinian sage and oregano. *Food Chem* 2011; **126**: 121-126.

[51]Rakholiya K, Kaneria M, Chanda S. Vegetable and fruit peels as a novel source of antioxidants. *J Med Plants Res* 2011, **5**, 63-71.

[52]Staszewski M, Pilosof MR, Jagus RJ. Antioxidant and antimicrobial performance of different Argentinean green tea varieties as affected by whey proteins. *Food Chem* 2011; **125**: 186-192.

[53]Coelho de Souza G, Hass APS, Von Poser GL, Schapoval EES, Elisabetsky E. Ethanopharmacological studies of antimicrobial remedies in the south of Brazil. *J Ethnopharmacol* 2004; **90**: 135-143

[54]Oliveira I, Sousa A, Morais JS, Ferreira ICFR, Bento A, Estevinho L, et al. Chemical composition, and antioxidant and antimicrobial activities of three hazelnut (*Corylus avellana* L.) cultivers. *Food Chem Toxicol* 2008; **46**: 1801-1807.

[55]Chanda S, Nair R. Antimicrobial activity of Polyalthia longifolia (Sonn.) Thw. var. *pendula* leaf extracts against 91 clinically important pathogenic microbial strains. *Chinese Med* 2010; **1**: 31-38.

[56]Tenore GC, Ciampaglia R, Arnold NA, Piozzi F, Napolitano F, Rigano D, et al. Antimicrobial and antioxidant properties of the essential oil of *Salvia lanigera* from Cyprus. *Food Chem Toxicol* 2011; **49**: 238-243.