Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2014; 6(2): 174-178

ISSN- 0975 1556

Research Article

Antioxidant and Antibacterial Properties of *Manilkara zapota* (L.) Royen Flower

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Available online: *1st April 2014*

ABSTRACT

The present study was aimed at evaluating the antioxidant and antibacterial properties of *Manilkara zapota* flower. The antioxidant property of the methanol and aqueous extract was determined by DPPH radical scavenging assay. The extracts showed excellent antioxidant activity with the IC_{50} values 1.97 g/ml and 4.22 g/ml for methanol and aqueous extract respectively. The antimicrobial property of the flower was studied by agar well diffusion method against few pathogenic organisms. Both the extracts exhibited significant antibacterial activity against all the four tested bacterial strains with zones of inhibition ranging from 26 mm to 29 mm. A qualitative analysis for the identification of the phytochemicals revealed the presence of phenols, tannins, quinones, alkaloids, flavones and saponins in the extracts which might have acted synergistically leading to its strong antioxidant and antibacterial efficiency. Hence, the methanol and aqueous flower extracts of *Manilkara zapota* can be considered as an interesting and economic source of natural antioxidant and antibacterial agent for utilization in nutraceutical and pharmaceutical industries.

Keywords: *Manilkara zapota*, Flower extract, Agar well diffusion, DPPH radical, Oxidative stress, Phytochemicals, Antimicrobial

Introduction

Medicinal herbs constitute an effective source of traditional and modern medicine. Many herbal remedies individually or in combination have been recommended in curing different diseases. The World Health Organization (WHO) estimated that about 80% population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs [1]. *Manilkara zapota* (L.) Royen, has been recognized in traditional medicine for its medical importance [2]. *M.zapota* commonly known as sapodilla is a plant belonging to the family Sapotaceae. The plant has been used in the indigenous system of medicine for various ailments. The different parts of the plant such as the leaves [3], bark [4] and seeds [5] have been extensively studied for antimicrobial activity. The leaves have also been established to contain antioxidant activity [6]. However, a search on the literature did not show any such activity in the flower of this plant. The flower has been traditionally used to relieve pulmonary complaints and fever. In order to extend our knowledge on the plant, this work was aimed to evaluate the antibacterial and antioxidant property of the methanol and aqueous flower extracts of *M.zapota*.

Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids [7]. Recent evidences have proved oxidative stress as being involved in the pathogenesis and progression of various diseases and hence have attracted much attention of the general public on the role of antioxidants in maintaining health and in prevention and treatment of diseases [8]. Antioxidants have the ability to protect human system from damage caused by free radical-induced oxidative stress [9]. Apart from this, the recent years have also brought an alarming rise in the prevalence of resistance of antibiotics to certain microorganisms [10]. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. Infectious diseases caused by bacteria, viruses, fungi and other parasites are major causes of death, disability, and social and economic disruption for millions of people [11]. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants [12]. A large number of populations in the developing countries still depend on traditional medicine for their health care needs due to its affordability, accessibility and cultural importance. In this present scenario, plants being a store-house of many bioactive compounds which are natural in origin and are known to be safe for consumption have prompted us to undertake the current study in order to identify both the properties from the flower extract of *M.zapota*.

MATERIALS AND METHODS

Plant collection: The flowers of *M.zapota* were collected from a garden in Ussoor, Tamilnadu, India. The plant was authenticated by a Botany Professor, Voorhees College,

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Table 1: Phytochemical analysis of methanol and aqueous extracts of *M.zapota* flower

Compound	Methanol extract	Aqueous extract
Phenols	+++	+++
Reducing sugars	+++	+
Carbohydrates	++	++
Flavones	+++	+
Glycosides	-	-
Saponins	+	+++
Steroids	-	-
Alkaloids	++	+++
Anthraquinones	-	+
Quinones	++	++
Proteins	+++	+++
Amino acids	-	-
Tannins	+++	+++
Phlobotannins	-	-

'+' indicates presence (+ - mild, ++ - moderate, +++ - high); '-' indicates absence

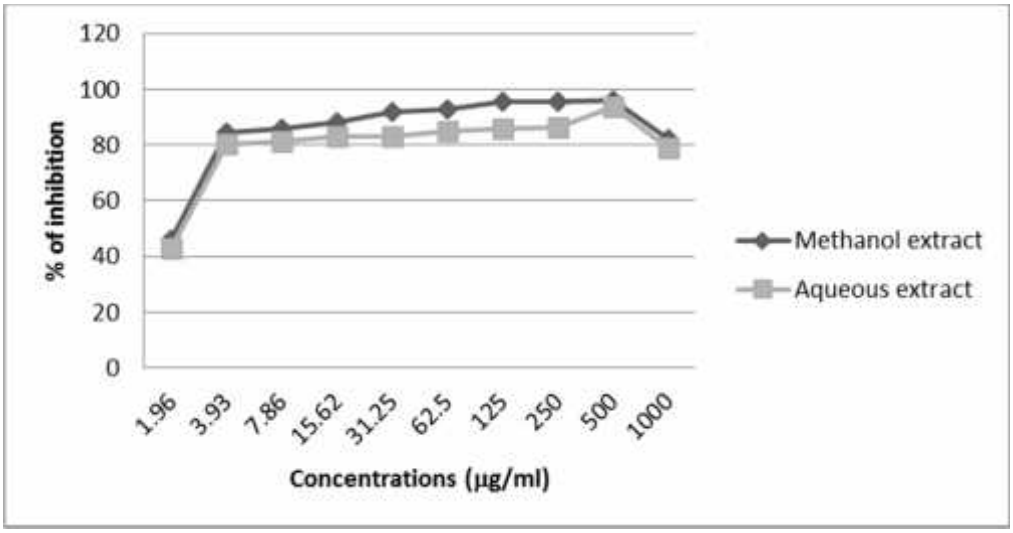

Figure 1: DPPH scavenging activity of methanol and aqueous extracts of M.zapota flower

Vellore. The collected flowers were washed thoroughly with running water, dried under shade in room temperature and coarsely powdered in a blender.

Plant extraction: Extraction was performed by cold maceration method. About 100 g of the powdered flower was soaked separately in methanol (500 ml) and distilled water (500 ml) for 4 days to obtain methanol and aqueous extract respectively. The whole mixture was filtered and the filtrate was transferred to a china dish kept in a water bath at 50°C to concentrate to dryness. The residual extract *M.zapota* methanol extract (MZME) and *M.zapota* aqueous extract (MZAE) were stored in air-tight containers until it was used. The extract yield (%) was calculated.

Extract yield (%) = (weight of the extract / weight of the raw material) x 100

Qualitative phytochemical analysis: About 500 mg of MZME and MZAE was weighed and dissolved in 25 ml of their respective solvents and subjected to qualitative phytochemical analysis to identify compounds such as phenols, reducing sugars, flavones, glycosides, saponins, alkaloids, anthraquinones, proteins and tannins following standard protocol [13].

In vitro antioxidant activity: The antioxidant activity of the extracts was determined by DPPH radical scavenging method [14] with minor modifications. About 10 l of each concentration (10 concentrations in serial dilution from 1000 g/ml) was added to 190 l of DPPH solution. After vortexing, the mixture was incubated for 20 min at 37°C. The control blank contained the solvent without the extract. The absorbance was measured at 517 nm. The IC_{50} values were determined using Graph Pad Software version 5.03. The experiment was done in triplicates and the percentage inhibition was calculated.

Inhibition (%) = [(OD of control – OD of test) / OD of control] x 100

Microorganisms and culture media: Four bacterial strains namely: *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Salmonella typhi* were collected from Microbial Type Culture Collection (MTCC), Chandigarh. Nutrient agar media were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India.

Antibacterial screening: The antibacterial activity of MZME and MZAE were determined by agar well diffusion method [15]. Plates were prepared by pouring Mueller Hinton Agar into sterile petridishes. The solidified plates

Table 2: Antibacterial sensitivity of methanol and aqueous extracts of *M.zapota* flower

Bacteria	Zone of inhibition (mm)		
	Methanol extract	Aqueous extract	Ciprofloxacin
Bacillus subtilis	29.0 ± 0.5	26.5 ± 0.5	30.0 ± 0.6
Staphylococcus aureus	28.5 ± 1.5	28.0 ± 1.0	32.5 ± 0.5
Salmonella typhi	27.5 ± 1.0	28.5 ± 1.4	31.5 ± 0.8
Pesudomonas aeruginosa	28.0 ± 1.5	26.0 ± 0.5	29.0 ± 1.0

Values are expressed in mean ± SD. The data are in triplicates.

after 10 min were swapped with bacterial cultures. Three wells of 5 mm diameter were bored each for MZME, MZAE and standard antibiotic ciprofloxacin. The samples were pipetted using sterile micropipette. The plates were then incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the zones of inhibition.

RESULTS

Extract yield: The percentage of extract yield varied with the solvents used. It was found to be 15.98% w/w and 9.39% w/w, for methanol and aqueous extracts respectively.

Phytochemical analysis: The qualitative phytochemical analysis indicated the presence of phenols, reducing sugars, carbohydrates, flavones, saponins, alkaloids, quinones, proteins and tannins in methanol extract and phenols, reducing sugars, carbohydrates, flavones, saponins, alkaloids, quinones, anthraquinones, proteins and tannins in the aqueous extract. However, the extracts did not contain glycosides, steroids and phlobotannins (Table 1).

Antioxidant acitivity: The methanol and aqueous extract of *M.zapota* flower was screened for its antioxidant activity using DPPH radical scavenging method. The methanol extract showed higher scavenging activity than the aqueous extract. At 1.96 g/ml concentration, the methanol extract showed 45.71% inhibition and the aqueous extract showed 42.75% inhibition. Following which, methanol and aqueous extract exhibited a significant dose-dependent increase in the percentage of inhibition with maximum scavenging of 96% and 93.49% respectively at 500 g/ml (Figure 1). The IC_{50} values were determined and found to be 1.97 g/ml for MZME and 4.22 g/ml for MZAE.

Antibacterial activity: *In vitro* antimicrobial activity of both the extracts of *M.zapota* flower was studied against four pathogenic bacteria. Both the extracts were effective against all the tested bacteria (Table 2). The zone of inhibition of methanol extract was the highest for *B.subtilis* (29 mm), followed by *S.aureus*, *P.aeruginosa* and *S.typhi* (27.5 mm). With the aqueous extract, *S.typhi* (28.5 mm) showed maximum zone of inhibition followed by *S.aureus*, *B.subtilis* and *P.aeruginosa* (26 mm).

DISCUSSION

Screening of herbal medicines has led to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases. Natural products have been shown to be a potential source of antioxidant and anti infective agents [16]. Secondary metabolites of plants have gained interest in the recent years. The phytochemical screening of the extracts confirmed the presence of several bioactive compounds like phenols, flavonoids, tannins and alkaloids which are natural compounds possessing a wide range of pharmacological activities. The compounds that are found in a particular extract depend on the solvent used. Since methanol and water are polar solvents, both the extracts showed the presence of more or less similar compound.

The antioxidant property of *M.zapota* confirms the presence of a new natural source of antioxidant since the percentage of inhibition exerted by the plant was statistically significant. DPPH is a commonly used substrate for fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay [17]. The colour change of DPPH solution from purple to yellow as the radical is quenched by the antioxidant [18] can be measured quantitatively by spectrophotometer at 517 nm. The scavenging potential of the extracts can be attributed to the presence of phenols, flavonoids and tannins [19]. This is supported by previous findings of antioxidant activity of *M.zapota* leaves [6] which has been correlated to the phenol content in the acetone extract [20]. Phenolic compounds such as flavonoids and tannins possess an ideal structure for the scavenging of free radicals [21]. Phenolic compounds are good electron donors and could terminate the radical chain reaction by converting free radicals to more stable products [22]. This activity depends on the structure of the molecules, and the number and position of the hydroxyl group in the molecules. On the other hand, *in vitro* studies have shown that flavonoids can directly scavenge molecular species of active oxygen which includes superoxide, hydrogen peroxide, hydroxyl radical, singlet oxygen or peroxyl radical. Thus the plant can be explained for their antioxidant activity owing to the presence of flavonoids [23]. The scavenging activity of flavonoids is primarily associated with the -ring catechol group [24] and resides mainly in their ability to donate electrons or hydrogen atoms [25].

Secondly, *M.zapota* flowers have also proved to be an effective antimicrobial. This property can also be attributed to the phenolic compounds such as flavonoids and tannins present in high amount in both methanol and aqueous extracts [26]. The lesser efficiency of the methanol extract towards gram-negative bacteria than the aqueous extract may be due to less amount of hydrophilic compounds in the methanol extract [27]. Earlier studies have reported that the ethyl acetate extract of *M.zapota* bark exhibited antibacterial activity due to the presence of flavonoid [3]. Phenolic compounds such as coumarin and quercetin had extended protection to gastroenteritis

disease-causing microbes [28]. Condensed tannins from *Rhizophora apiculata* demonstrated high antibacterial activity and also showed higher percentage of DPPH scavenging activity [29]. Inhibitory mechanism of tannins is explained as direct inhibition caused by interacting with the membranes of cell walls or extracellular proteins [30]. The property to precipitate proteins is eponymous for tannins and they are unlikely to penetrate bacterial membrane and thus the tannins and flavonoids in *M.zapota* methanol and ethanol extract are expected to have preferentially interacted with the cell wall proteins or membrane proteins [31]. This facilitates their use as antimicrobials in food as well. However, additionally the flowers of *M.zapota* have to account for beneficial or adverse effects on human health.

CONCLUSION

In conclusion, we found that the methanol and aqueous flower extracts of *M.zapota* exhibited significant DPPH radical scavenging activity and antimicrobial effect. The higher antioxidant and antimicrobial activities observed may be due to the presence of phytocompounds such as phenols, flavonoids, tannins, alkaloids, saponins and quinones. Being a crude extract, there is a possibility for the compounds to have acted in additive or synergistic action. Hence, our data suggest that *M.zapota* flower extract could be utilized to develop natural antioxidant and antimicrobial agents. However, further studies on these extracts with respect to antioxidant properties *in vivo* are needed.

ACKNOWLEDGEMENTS

The authors thank the Biomedicine Lab, Voorhees College, a UGC-sanctioned Major Research Project for providing lab facilities to undertake this study.

Conflict of interest: We declare that we have no conflict of interest.

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