Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Issue 3, 2012 ISSN - 0974-2441

Research Article

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF GREEN AND RIPE FRUITS OF *AVERRHOA CARAMBOLA* LINN. AND *ZIZYPHUS MAURITIANA* LAM.

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Received: 10 March 2012, Revised and Accepted: 15 May 2012

ABSTRACT

Consumption of diets rich in fruits, vegetables and derived food products can bring substantial health benefits. Research interest thus has increased in natural antioxidants and antimicrobials present in herbs, fruits or vegetables. Underutilized tropical fruits of India provide limitless opportunities for screening of novel drugs. Present study was aimed to understand the antimicrobial and antioxidant activities of tropical fruits *Averrhoa carambola* Linn. (starfruit) and *Zizyphus mauritiana* Lam. (jujube) fruits. The edible parts of the fruits were analyzed for different phytochemicals and phenolics, flavonoids, alkaloids and glycosides were found in all ripe and green starfruits or jujubes. Green fruits of *Averrhoa carambola* showed better antimicrobial activities in comparison with ripe varieties. Widest inhibition zones (14-15 mm DIZ) were seen in cases of extracts of ripe *Zizyphus mauritiana* against *Escherichia coli* and *Staphylococcus aureus.* Common antioxidants like Phenolics, flavonoids and ascorbate were measured. Extracts of ripe fruits contain higher amounts of flavonoids and ascorbate. Bers contain higher amount of ascorbate than starfruits. Ripe jujube or *Zizyphus mauritiana* extract showed strongest free radical scavenging or antioxidant activity among the tested.

Keywords: *Averrhoa carambola, Zizyphus mauritiana,* antimicrobial, antioxidant, phenolics, free radical.

INTRODUCTION

Plants have always been a significant source of natural products having therapeutic potential. Fruits, vegetables, nuts and grains are accounted to have many important biological effects including antioxidant, antitumor, antimutagenic and antimicrobial properties (Guanghou and Lai, 2002). These natural plant products are medically helpful and least detrimental with very few side effects as compared to synthetic medicines. Many such compounds have been isolated from different plant sources and applied into clinical medical practice.

It is well recognized that consumption of diets rich in fruits, vegetables and derived food products can bring substantial health benefits. A diet rich in vegetables and fruits may provide protection against different diseases like cardiovascular disease, chronic disease and certain types of cancer (Farrukh et al., 2006; Ness and Powles, 1997, Steinmetz and Potter, 1996). This has attracted a great deal of research interest in natural antioxidants and antimicrobials present in herbs, oilseeds, fruits and vegetables. Importance of the antioxidant based drugs or formulations for the prevention and treatment of several diseases was reported by Spigno and De Faveri (2007). The principal agents responsible for these protective effects are the antioxidant substances which are present in considerable high amount in such plant products. These antioxidants can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species (ROS) including reactive free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl and non- radicals such as hydrogen peroxide, hypochlorous, etc. Antioxidants scavenge free radicals by inhibiting initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, superoxide and singlet oxygen (Lim et al., 2007; Phapale and Misra-Thakur, 2010).

In recent decades, antibiotic resistance is a rising problem worldwide (Cohen, 2002; Walsh, 2000). This has lead to the search for new, safe and effective antimicrobial agents from alternative natural resources like plant products. At the same time, there is a growing demand among consumers for natural preservative or additives in processed foods (Gutierrez et al., 2008). In comparison to chemical or synthetic additives herbal additives are preferred as these are safer, flavour enhancer and without any side effects (Brull and Coote, 1999). Herbal extracts are fast becoming popular as natural antimicrobial preservatives or additives (Cox et al., 2010; Akarpat et al., 2008; Pazos et al., 2008). Antibacterial activities of extracts of different plants against various microorganisms have been reported by many scientists (Chaudhury and Tariq, 2008;Hussain et al., 2010; Shan et al., 2007; Nair and Chanda, 2006). Some medicinal herbs have also been assessed (Ahmad and Beg, 2001).

In India, rich resource of wild or underutilized fruits is available. These underutilized fruits have recently drawn attention of many researchers as a natural source of treatment for curing various diseases. Some studies on underutilized fruits have claimed them to be better sources of nutrients (Phapale and Misra-Thakur, 2010). Such underutilized tropical fruits provide limitless opportunities for screening of novel drugs.

Five-lobbed fleshy, yellow-greenish, edible fruits of *Averrhoa carambola* Linn. (starfruit) of Oxalidaceae is native of South-East Asia and cultivated in some parts of India. The fruits are good source of antioxidants and used traditionally in mouth ulcers, toothache, nausea, diarrhea, ascites etc. (Shui and Leong, 2006). Edible fruits of *Zizyphus mauritiana* Lam. (jujube or ber) of Rhamnaceae, an evergreen perennial tree native to India is used for various ethnomedical uses in asthma, burning, boils, nausea, vomiting, piles etc (Karon et al., 2011; Mahajan and Chopda, 2009).

Hence the present study has been aimed to understand the antimicrobial and antioxidant activities of tropical *Averrhoa carambola* Linn. and *Zizyphus mauritiana* Lam. fruits, which are still underutilized.

MATERIALS AND METHODS

Plants collection and preparation

Green and ripe mature fruits of *Averrhoa carambola* and *Zizyphus mauritiana* Lam. were collected in December 2011, from markets of Kolkata, West Bengal, India. The fruits were cleaned and deseeded to make these completely free from any possible contamination. The edible portions of the fruits were dried at 50 ºC for 3-4 days, then separately ground into fine powder using a mechanical grinder and sieved. The powder was kept in dark coloured glass bottles and subsequently used.

Preparation of solvent extraction

40 g of each dry powder was mixed in 100 ml sterile ethanol (100%) for 48 hours at 24ºC with stirring (Liu and Nakano, 1996). The extracts were centrifuged and filtered through Whatman No.1 filter paper and bacterial 0.45µm filter (Millipore). Then extracts were evaporated using vacuum rotary evaporator to near dryness and stored in glass vials in dark at 4ºC. Extractive values were calculated in terms of percentage considering the weight of plant material as 100%. These crude solvent extracts were diluted with either sterile

double distilled water or 10% dimethyl sulphoxide (DMSO) which are to be used as negative control respectively to obtain required concentration before experiments.

Phytochemical evaluations

The extracts were tested to phytochemical evaluation using standard techniques of plant secondary metabolites according to Harborne and Turner (1984) and Trease and Evans, (2002). Extracts were tested for phenolics, tannin, flavonoids, alkaloids, triterpenoids, saponin, steroids, coumarin, anthraquinone and glycosides.

Test micro-organisms

Four enteropathogenic, three food-spoiler and one probiotic bacterial strains were selected for the antimicrobial activities of these fruits study. The strains used were *Salmonella enterica* serovar typhimurium MTCC 3224, *Serratia marcescens* MTCC 4822, *Staphylococcus aureus* MTCC 7405, *Escherichia coli* MTCC 3221, *Klebsiella pneumoniae* subsp. pneumoniae MTCC 6644, *Proteus vulgaris* MTCC 7299, *Bacillus cereus* MTCC 6909, *Lactobacillus brevis* MTCC 4460 and those strains were obtained from MTCC, IMTECH, Chandigarh, India. All bacterial cultures were maintained on tryptic soy agar (HiMedia) and subcultured regularly. The fungal strain *Aspergillus niger* was taken from laboratory collection (isolated from bread) and grown on Sabouraud dextrose agar (HiMedia). Standard inoculum was prepared by sub-culturing 4-5 freshly grown isolated colonies of each strain in Tryptic soy broth (TSB) and incubated at 35-37 ºC for 24 hours. Inocula were standardized with sterile TSB to give final cell load of 10^6-10^7 CFU/ml.

Disc diffusion bioassay

The disc diffusion test was performed as described by Jorgensen et al (1999). A 0.5 ml standardized inoculum suspension of each bacterial strain was spread on TSA plates with a sterile bent glass rod spreader. Sterile 6-mm Whatman no.1 filter paper discs were aseptically placed on plates. Sample decoctions or extracts of standard concentrations (10 mg dry weight) were aseptically poured on the discs along with sterile double distilled water or 10% DMSO as negative and ampicillin as positive controls. Plates were allowed to stand for 30 minutes at room temperature prior to incubation at 35-37 ºC for 24 hours. The inhibition zone diameters were measured three times and means were represented.

Total phenolic Content (TPC)

The total phenolic contents of extracts were determined spectrophotometrically (Singleton and Rosi, 1965). One ml of Folin-Ciocalteu's reagent (Merck, India), previously diluted (1:20), was added to one milliliter of sample (250 µg/ml) and mixed thoroughly. To the mixture, 4 ml of sodium carbonate (75 g/L) and 10 ml of distilled water were added and thoroughly mixed. The mixture was allowed to stand for 2 h at room temperature. Contents were then centrifuged at 2000 g for 5 min and the absorbance of the supernatant was taken at 760 nm. A standard curve was obtained using various concentrations of gallic acid (Sigma-Aldrich, Germany). Results were expressed as percentage of gallic acid equivalents (GAE) per 100 g of fresh mass. The total phenolic compounds (TPC) was expressed as gallic acid equivalents (GAE)/ per g of dry weight (mgGAE/g dw) using the following equation obtained from a standard gallic acid (Sigma-Aldrich) graph y= 3.9207x + 1.0607 (R^2 =0.9932).

Total flavonoid content

Total flavonoid content was measured by aluminum chloride colorimetric assay (Marinova et al., 2005). 1ml of sample or standard solution of catechin (500 µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added. After 5 minutes, 2 ml of 1 M NaOH was added and total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared control at 510 nm. Total flavonoid content of the sample was expressed as percentage of catechin equivalent per g dry weight (mgCE/g dw).

Ascorbic Acid Content

Ascorbic acid content in the juice was determined with DCPIP visual titration method described earlier by Ranganna (2001). The DCPIP dye, which is blue in alkaline solution, is reduced by ascorbic acid to a colorless form. About 5 mL of extract solution was titrated with DCPIP dye to a pink color end point. The ascorbic acid content was determined by the titration of the standard ascorbic acid solution with DCPIP dye.

DPPH free radical scavenging activity

The antioxidant or freeradical scavenging activity of the extracts was measured on the basis of decrease in the absorbance of methanol solution of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Sreejayan and Rao, 1996). DPPH is one of the few stable and commercially available organic nitrogen radicals (Huang *et al*., 2005) exhibiting a dark purple color at absorbance 517 nm. When free radicals are scavenged, DPPH will be reduced, producing a light yellow coloration reducing the absorbance. 05 ml of DPPH (25 mg /L) solution was added to 1 ml of sample solution (at different concentrations). Mixture was shaken vigorously and kept at room temperature for 30 min in dark. Then the absorbance was measured at 517 nm and compared with standards. Scavenging activity was calculated as the percentage inhibition (I%) using the following formula:

$$\% \text{ DPPH Anti-radical activity (I\%)} = \frac{\textbf{(}\text{Control Absorbance - Sample Absorbance}\textbf{) x 100}}{\text{Control Absorbance}}$$

Radical-scavenging potential was expressed as IC50 value (calculated from linear regression of the graph of concentration vs. I%) representing the concentration, which scavenged 50% of the DPPH radicals.

Statistical analyses

For all tests three replicates were done and the mean values and standard deviations were determined.

Table 1: Phytochemical qualitative evaluation of green and ripe fruits of *Averrhoa carambola* and *Zizyphus mauritiana* .

Phytochemicals	*Averrhoa carambola*		*Zizyphus mauritiana*	
	Green	Ripe	Green	Ripe
Phenolics	+	+	+	+
Tannin	+	+	+	-
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Triterpenoids	-	-	+	+
Saponin	+	-	+	+
Steroids	+	+	+	-
Coumarin	-	-	-	-
Anthraquinone	+	+	-	-
Glycosides	+	+	+	+

RESULTS AND DISCUSSION

The edible parts of the fruits were analyzed for different phytochemicals (Table 1). The phenolics, flavonoids, alkaloids and glycosides were present in all ripe and green starfruits or jujubes. Tannins, steroids, anthraquinones were present in both types of starfruits. Coumarin was absent in all cases. Flavonoids,

triterpenoids and saponin were present in both types of jujubes or bers. While anthraquinone was absent in all types of jujubes, tannin and steroid were absent in ripe jujubes. Saponin was found in only green starfruits. Edible part of ber fruit was recently found to be rich in phenolic phytochemicals, with naringeninas a major flavonoid and *p*-coumaric acids as predominant phenolic acids including a steroid β-sitosterol (Memon et al., 2012).

Table2: Antibacterial activities, indicated by diameter of inhibition zone (DIZ, mm, for 10 mg dry wt./ disc, Mean±SD) of selected samples against the micro-organisms [- means <6mm DIZ i.e DIZ of negative control]

Fruits	Types	*E.coli*	*S.aureus*	*S. enterica*	*S. marce-scens*	*K. pneu-moniae*	*P.vulgaris*	*B. cereus*	*L.brevis*	*A.niger*
	Green	10.67±	10.67±	-	7.33±	11±1	10.33±	12±1	8.33±	8±2
Averrhoa		1.527	0.577		0.577		1.527		0.577	
caramboa	Ripe	8.67±	7±1	6.67±	9.33±	9.67±	7.33±	9.33±	-	-
		0.577		0.577	0.577	0.577	1.154	0.577		
	Green	12.33±	14±1	9.67±	8.33±	10±1	9.33±	7.67±	10.67±	10.67±
Zizyphus		1.527		0.577	1.154		0.577	0.577	0.577	3.055
mauritiana	Ripe	14.33±	15.33±	7.67±	12±1	12.67±	-	13±1	6.67±	7.67±
		1.527	0.577	1.527		1.527			0.577	0.577

The disc diffusion assay showed that the fruit extracts have different degrees of bacterial and fungal growth inhibition, depending on the strains (Table 2). Green fruits of *Averrhoa carambola* showed better antimicrobial activities in comparison with ripe varieties. Ripe starfruit extract was not very effective against fungus *Aspergillus niger* and bacteria like *Lactobacillus brevis, Salmonella enterica, Staphylococcus aureus, Proteus vulgaris.* But ripe fruits of *Zizyphus mauritiana* showed stronger antimicrobial activities in comparison with green varieties. Widest inhibition zones (14-15 mm DIZ) were seen in cases of extracts of ripe *Zizyphus mauritiana* against *Escherichia coli* and *Staphylococcus aureus.* The fungus *Aspergillus niger* was found less sensitive against these fruit extracts. *Zizyphus nummularia* found to have potential antibacterial and antifungal activity against four medically important bacterial and fungal strains (Gautam et al., 2011).

Table 3: Phytochemicals estimation and antioxidant activities of fruit extracts

Phytochemicals	*Averrhoa carambola*		*Zizyphus mauritiana*	
	Green	Ripe	Green	Ripe
Phenolics (mgGAE/g dw)	112±20	98±12	104±16	124±23
Flavonoids (mgCE/g dw)	2.48±0.06	3.87±0.11	2.52±0.06	3.75±0.09
Ascorbate (mg/g dw)	58±4	72±6	78±4	93±8
DPPH IC_{50} (mg/ml)	8.25	7.65	7.50	6.15

Phytochemicals estimation and antioxidant activities of fruit extracts were shown in Table 3. TPC results were expressed as mg gallic acid equivalent as this compound represents the most simple form of a phenolic compound. While ripe starfruits had lesser amount of phenolics, ripe jujubes or bers contained higher amounts of phenolics. Extracts of ripe fruits contain higher amounts of flavonoids and ascorbate. Bers contain higher amount of ascorbate than starfruits. The phenolic compounds of starfruit were identified as epicatechin, gallic acid, gallotannin, proanthocyanidins etc. (Shui and Leong, 2006). Proanthocyanidins, which existed as singly-linked dimers through pentamers, were most likely to be the major antioxidants in star-fruit (Shui and Leong, 2006). Theasinensin A, a polyphenol related to antimicrobial and antioxidant activities was isolated from fruits of *Z. jujube* (Tsutomo et al., 2005).

DPPH scavenging assay is applied extensively for the determination of free radical scavenging or antioxidant activity of any compound. DPPH assay measures the capability of the extract to donate hydrogen to the radical. In DPPH assay the lower the IC50 the better it is able to scavenge the radicals, particularly peroxy radicals which are the propagators of the autoxidation of lipid molecules and thereby break the free radical chain reaction (Frankel, 1991). Ripe starfruit showed better antioxidant activity. Ripe jujube or *Zizyphus mauritiana* extract showed strongest antioxidant activity among the tested. Even the green variety possesses higher antioxidant activity than ripe or green starfruits.

CONCLUSION

It could be concluded that starfruit and jujube have potentially good antimicrobial and antioxidant efficacy. The tested extracts of ripe or green fruits have shown antimicrobial efficacy against most of microbes examined. Ripe jujube or *Zizyphus mauritiana* extract showed strongest antioxidant activity. Differential antimicrobial or antioxidant activity of extracts against different bacteria might be due to present of different active phyto-chemicals. Among those antimicrobial compounds , phenolic compounds , terpenoids , and alkaloids are very important compounds in antimicrobial or antioxidant effects. Further study is required to determine the different active compounds from these under-utilized tropical fruits and their full spectrum of efficacy. These resources have the prospect of finding new clinically efficient antimicrobial or antioxidant compounds and the knowledge can be extended for future investigation into the field of pharmacology for better drug discovery.

ACKNOWLEDGEMENT

This work has been financially supported by University Grants Commission, India. Author is thankful to MTCC, IMTECH, Chandigarh from where necessary strains were obtained. Author is also thankful to University of Calcutta and Bose Institute for providing laboratory facilities.

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