Cytotoxicity, Platelet Aggregation Inhibitory and Antioxidant Activity of *Curcuma amada* Roxb. Extracts

Rudragoud S. Policegoudra[1]*, Raghu H. Chandrashekhar*[2], *Somaradhya M. Aradhya*[3]
and Lokendra Singh[1]

[1]Defence Research Laboratory, 784001 Tezpur, Assam, India
[2]Manipal College of Pharmaceutical Sciences, Manipal University, 576104 Manipal, Karnataka, India
[3]Central Food Technological Research Institute, 570020 Mysore, Karnataka, India

Summary

Mango ginger (*Curcuma amada* Roxb.) is a unique spice having morphological resemblance to ginger but imparts raw mango flavour. The sequential extraction of mango ginger rhizome powder was carried out using hexane, chloroform, ethyl acetate, acetone, methanol and water. The phenolic content was the highest in methanol extract, followed by acetone, ethyl acetate and water extracts. Among these, chloroform extract exhibited high lipid peroxidation inhibitory activity and metal chelating activity, whereas ethyl acetate extract showed high DPPH radical scavenging activity and superoxide radical scavenging activity. Mango ginger extracts also showed potential platelet aggregation inhibitory activity and cytotoxicity properties.

Key words: *Curcuma amada*, mango ginger, antioxidant activity, phenolics, platelet aggregation inhibitory activity, cytotoxicity

Introduction

Historically, plant-derived extracts have been considered as an important remedy for maintaining health, enhancing overall immune status, and prevention and treatment of chronic diseases (*1*). Extracts of aromatic herbs, spices, and medicinal plants are employed in food processing to impart flavour and other functional properties. The naturally occurring phytochemicals present in fruits, vegetables and spices can prevent or reduce the risk of many diseases (*2*). The presence of polyphenols, terpenoids, alkaloids, flavonoids and other secondary metabolites in plants will provide a scientific validation for their popular use (*3,4*).

The bioactive properties of phenolic compounds are mainly due to the result of various possible mechanisms like free radical scavenging activity, transition metal chelating activity, inhibition of lipid peroxidation and platelet aggregation (*5–8*). Plant extracts are also an alter-native to the currently used anti-platelet agents, because they constitute a rich source of bioactive chemicals (*9,10*). These potentially multiple mechanisms of antioxidant action make the diverse group of phenolic compounds an interesting target in the search for natural health-beneficial phytochemicals (*11*). There is numerous evidence that free radicals induce oxidative damage to lipids, proteins and nucleic acids, which eventually causes atherosclerosis, ageing, cancer, *diabetes mellitus*, inflammation, AIDS and several degenerative diseases in humans (*12–15*).

The genus *Curcuma* (Zingiberaceae) comprises over 80 species of rhizomatous herbs. They originate from the Indo-Malayan region and are widely distributed in the tropics of Asia to Africa and Australia (*16*). Mango ginger (*Curcuma amada* Roxb.) is a unique spice having morphological resemblance to ginger. The main use of mango ginger rhizome is in the manufacture of pickles, as a

*Corresponding author; Phone: ++91 3712 258 508; Fax: ++91 3712 258 534; E-mail: poligene@rediffmail.com

source of raw mango flavour for foods and for therapeutic purpose (*17*). Ayurveda, the oldest system of medicine in India, has given importance to rhizome as an appetizer, alexiteric, antipyretic, aphrodisiac and laxative (*17,18*). It is also used in treatment of biliousness, itching, skin diseases, bronchitis, asthma, hiccups and inflammation due to injuries (*18,19*). According to Unani systems of medicine, it is a diuretic, maturant, emollient, expectorant, antipyretic and appetizer (*19*). It is useful against inflammation in the mouth, ear, as well as gleet, ulcers on the male sex organs, scabies, lumbago and stomatitis (*18–20*). In spite of its strong medicinal properties and therapeutic uses in Ayurveda and Unani systems of medicine, there are few reports on the antioxidant activity, platelet aggregation inhibitory activity and cytotoxic properties of different extracts of mango ginger rhizome. The present investigation explores the antioxidant activity, cytotoxicity and platelet aggregation inhibitory potential of different mango ginger rhizome extracts.

Materials and Methods

Plant material

Fresh and healthy mango ginger rhizomes were procured from the local market, Mysore, India. Rhizomes were washed, sliced and dried in a hot air oven at 50 °C for 72 h and powdered to 60 mesh in an apex communiting mill (Apex Construction Ltd, London, UK).

Chemicals

Folin-Ciocalteu phenol reagent, potassium ferricyanide, 3-(2-pyridyl)-5,6-bis-(4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine) and ferric chloride were purchased from Sisco Research Laboratories (Mumbai, India). Gallic acid, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT), sulphorhodamine B (SRB) dye, Trizma base, collagen, nicotinamide adenine dinucleotide (NADH), dimethyl sulphoxide (DMSO), butylated hydroxyanisole (BHA), trichloroacetic acid (TCA) and newborn calf serum (NBCS) were purchased from Sigma-Aldrich (Sternheim, Germany). Nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), thiobarbituric acid (TBA), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India). All other reagents were of analytical grade and other chemicals used in this study were of highest purity.

Preparation of extracts

Sequential extraction was carried out with the mango ginger powder using solvents of different polarity in order to resolve the compounds of different polarity effectively and completely. About 100 g of dry mango ginger powder were sequentially extracted using *n*-hexane, followed by chloroform, ethyl acetate, acetone, methanol and water using orbital incubator shaker (Remi Instruments, Mumbai, India) at 100 rpm and room temperature ((25± 2) °C) under atmospheric pressure. After each solvent extraction step, the extracts were filtered and concentrated by using rotary evaporator (Büchi Rotavapor R-124,

Büchi Labotechnik AG, Flawil, Switzerland). The concentrated extracts were freeze-dried and stored in refrigerator until use.

Determination of phenolics

Total phenolic content was determined according to the modified method of Taga *et al.* (*21*). In brief, 100 µL of test samples (2 mg/mL solution) were mixed with 2 mL of 2 % sodium carbonate solution. After 3 min, 100 µL of 50 % Folin-Ciocalteu phenol reagent were added to the mixture. After 30 min of incubation at room temperature, absorbance was measured at 750 nm against the blank. Total phenolic content was calculated on the basis of the calibration curve of tannic acid used as standard reference.

Antioxidant activities of mango ginger extracts

Determination of DPPH˙ scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was carried out according to the methods described earlier (*22–24*). Briefly, 1 mL of DPPH˙ (500 µM in ethanol) was added to a mixture of test samples (10–100 µL) and 0.8 mL of Tris-HCl buffer (pH=7.4). After vigorous shaking, the mixture was allowed to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm with a UV-VIS spectrophotometer (UV-160A, Shimadzu Co, Japan). BHA was used as positive control. Radical scavenging potential was expressed as EC_{50} value, which represents the sample concentration at which 50 % of the DPPH˙ radicals were scavenged.

Determination of superoxide radical scavenging activity

The superoxide scavenging ability was assessed according to a modified method as described earlier (*24, 25*). The superoxide radicals were generated in 1 mL of Tris-HCl buffer (0.02 M, pH=8.3) containing NBT (0.1 mM), NADH (0.1 mM) and PMS (10 µM) with or without the sample. The colour reaction was recorded at 560 nm every 30 s for 2 min using UV-VIS spectrophotometer (UV-160A, Shimadzu Co). BHA was used as positive control. Radical scavenging potential was expressed as EC_{50} value.

Determination of lipid peroxidation inhibitory activity

Lipid peroxidation inhibitory activity was carried out according to the method of Duh and Yen as described earlier (*26*). In brief, lecithin (in 3 mg/mL of phosphate buffer, pH=7.4) was sonicated in a UP 50H ultrasonic processor (Hielscher Ultrasonics GmbH, Teltow, Germany). The test samples (100 µL) were added to 1 mL of sonicated lecithin, and then 10 µL of $FeCl_3$ (400 mM) and 10 µL of L-ascorbic acid (400 mM) were added to induce the lipid peroxidation. The reaction was stopped by adding 2 mL of 0.25 M HCl containing 15 % TCA and 0.375 % TBA, after incubation for 1 h at 37 °C. The absorbance of the supernatant was measured at 532 nm. BHA was used as positive control. Inhibitory activity was expressed as EC_{50} value, which is sample concentration at which 50 % of lipid peroxidation was inhibited.

Determination of metal chelating activity

The chelation of ferrous ions by the test sample was estimated by the method as described earlier (*27,28*).

Briefly, the test samples at different concentrations were mixed with 0.05 mL of 2 mM $FeCl_2$ and 0.2 mL of 5 mM ferrozine and the mixture was vigorously shaken. After 10 min, the absorbance of the mixture was read at 562 nm. EDTA was used as positive control. Results were expressed as EC_{50} value, which represents the sample concentration at which 50 % of metal chelation occurred.

Total reducing power determination

The reducing power was quantified by the method of Yen and Chen (29) with minor modifications as described earlier (24). Different concentrations of test samples (10–100 μL) in phosphate buffer (0.2 M, pH=6.6) were incubated with potassium ferricyanide (1 % by mass per volume) at 50 °C for 20 min. The TCA solution (10 % by mass per volume) was added to terminate the reaction and the mixture was centrifuged at 3000 rpm for 20 min. The supernatant was mixed with distilled water and ferric chloride (0.1 % by mass per volume) solution, and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Cytotoxicity of mango ginger extracts

Preparation of test solutions

For cytotoxicity studies, each extract was weighed separately, dissolved in 1 mL of distilled dimethyl sulphoxide (DMSO) and volume was made up to 10 mL with DMEM, pH=7.4, supplemented with 2 % inactivated NBCS (maintenance medium) to obtain a stock solution of 1 mg/mL concentration, sterilized by filtration and stored at –20 °C until use. Maintenance medium was used for dilution of extracts. Serial twofold dilution of the extracts was prepared from the stock solution to obtain lower concentrations. The mango ginger extract mass used for cytotoxicity assay ranged from 10 to 500 μg.

Cell lines and culture medium

Vero cell (normal African green monkey kidney) culture was procured from National Centre for Cell Sciences (NCCS), Pune, India, and A-549 (human small cell lung carcinoma) cells from Christian Medical College, Vellore, India. Stock cells of Vero and A-549 cell lines were cultured in DMEM medium supplemented with 10 % inactivated NBCS, penicillin (100 IU/mL), streptomycin (100 μg/mL) and amphotericin B (5 μg/mL) in a humidified atmosphere of 5 % CO_2 at 37 °C until they were confluent. The cells were dissociated with TPVG solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The stock cultures were grown in 25-cm^2 flat bottles and all experiments were carried out in 96-well microtitre plates.

Determination of mitochondrial synthesis by MTT assay

The MTT assay was carried out according to the method of Denizot and Lang (30). Briefly, the trypsinized monolayer cell culture was adjusted to 10^5 cells/mL using 10 % NBCS containing DMEM medium. The cell suspension (approx. 10 000 cells) was added to each well of the 96-well microtitre plate. After 24 h, the supernatant was aspirated, and the monolayer was washed with the medium and 100 μL of different extract concentrations were added to the cells in microtitre plates. The cells were in an exponential growth phase when the extracts were added to the culture. The plates were then incubated for 3 days in 5 % CO_2 atmosphere at 37 °C. After 72 h, 50 μL of MTT in DMEM-PR (Dulbecco's modified Eagle's medium with phenol red, 2 mg/mL) was added to each well. The plates were gently shaken and incubated for 3 h at 37 °C in 5 % CO_2 atmosphere. The absorbance was measured using a microplate reader at 540 nm. The percentage of growth inhibition was determined and CTC_{50} (cytotoxic concentration at which 50 % of the cells are dead) values were generated from the dose-response curves for each cell line.

Determination of total cell protein content by sulphorhodamine B (SRB) assay

The sulphorhodamine B assay was performed according to the method of Skehan et al. (31). The monolayer cell culture was trypsinized and the cell count was adjusted to 10^5 cells/mL using DMEM medium containing 10 % NBCS. To each well of the microtitre plate, about 0.1 mL of the diluted cell suspension (approx. 10 000 cells) was added. The plates were incubated at 37 °C for 3 days in 5 % CO_2 atmosphere. After 72 h, 25 μL of 50 % trichloroacetic acid were carefully added to the wells in such a way that it forms a thin layer over the extract to form an overall concentration of 10 %. The plates were aspirated and washed five times with Millipore water to remove the traces of the medium, extract and serum, and air dried. They were stained with SRB (0.4 % prepared in 1 % acetic acid, 50 μL/well) for 30 min. The plates were then air dried. Tris base (10 mM, 100 μL) was then added to the wells to solubilise the dye and shaken vigorously for 5 min. The absorbance was measured using microplate reader at a wavelength of 540 nm. The data were expressed as percentage of growth inhibition.

Determination of platelet aggregation inhibitory activity

Platelet aggregation inhibitory activity was determined according to the method of Policegoudra et al. (24). Briefly, platelet-rich plasma (PRP) was obtained by centrifugation of the citrated blood at 1100 rpm for 20 min, and platelet poor plasma (PPP) was obtained by centrifuging again the residual blood at 2500 rpm for 20 min. Aggregation was measured turbidimetrically in a Chrono-log dual channel aggregometer (Chrono-log Corp., Havertown, PA, USA) at 37 °C, with constant stirring at 1000 rpm. The aggregation was induced by collagen (10 μM) to 0.45 mL of PRP at 37 °C. Similarly, the mango ginger extract and PRP mixture was incubated for 5 min before induction with collagen (10 μM). The change in turbidity was recorded with reference to PPP using an omniscribe recorder for at least 5 min. The differences in the slope between the control and the treated sample were expressed as percent inhibition of platelet aggregation by mango ginger extracts.

Statistical analysis

The experiments were carried out in triplicates. Significant differences (p<0.05) were determined by Duncan's multiple range test (DMRT).
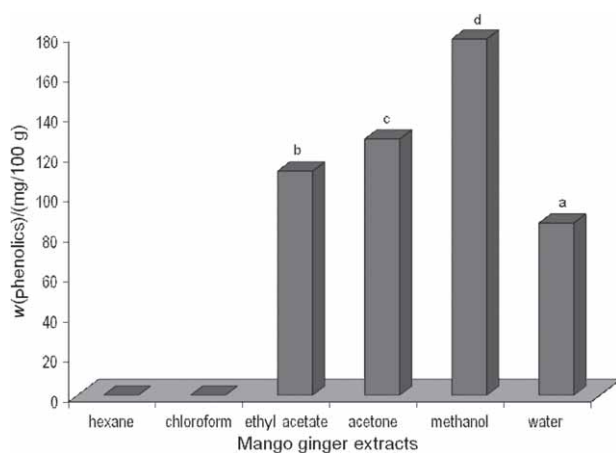
Results and Discussion

Preparation of mango ginger extracts

Different mango ginger extracts were obtained by sequential extraction with mango ginger powder using different solvents with increasing polarity. Sequential extraction of 100 g of mango ginger powder using hexane, chloroform, ethyl acetate, acetone, methanol and water yielded 11, 8, 0.9, 0.8, 10 and 5 g of extract, respectively. The purpose of employing sequential extraction with the same powder was to resolve all types of compounds with different polarity effectively and completely.

Phenolic content

Among six different extracts of mango ginger rhizome, the methanol extract showed high phenolic content followed by acetone, ethyl acetate and water extracts (Fig. 1). The phenolic compounds were not detected in hexane and chloroform extracts. Antibacterial activity of free and bound phenolics from mango ginger rhizomes has been reported by Siddaraju and Dharmesh (*32*). The free phenolic acids present in mango ginger were caffeic (26 %), gentisic (24 %), and ferulic (20 %), followed by gallic (10 %), cinnamic (7 %), protocatechuic (7 %), and small amounts of syringic (4 %) and *p*-coumaric (2 %) acids. Mango ginger also contains bound phenolic compounds like ferulic (47 %), cinnamic (29 %), *p*-coumaric

(11 %), syringic (5 %), caffeic (4 %), vanillic (2 %), gallic (1 %), and gentisic (1 %) acids. They demonstrated that both free and bound phenolic fractions of mango ginger were effective in inhibiting H^+/K^+-ATPase activity and *Helicobacter pylori* growth. Cinnamic and ferulic acids present in phenolic fractions of mango ginger contribute significantly to H^+/K^+-ATPase as well as to *H. pylori* growth inhibition. Plant phenolics have received considerable attention because of their potential antioxidant activity (*33*).

Antioxidant activity of mango ginger

DPPH radical scavenging activity

Except hexane and chloroform extracts, all other mango ginger extracts showed DPPH· scavenging activity. Ethyl acetate extract exhibited potential radical scavenging activity with very low IC_{50}, while acetone, methanol and water extracts showed less activity with high IC_{50} values (Table 1). The high antioxidant activity of ethyl acetate extract may be due to the cumulative effect of potential phenolic compounds. The concentration of phenolic compounds was lower in ethyl acetate extract compared to other extracts, but they still might have radical scavenging properties. Phenolic compounds are vital plant constituents with potential radical scavenging ability due to the presence of hydroxyl groups (*34,35*). DPPH radical scavenging activity of mango ginger extracts may be attributed to the presence of hydrogen-donating ability of -OH and -CH$_3$ groups in antioxidant compounds (*36,37*).

Superoxide radical scavenging activity

Among six extracts of mango ginger, only ethyl acetate and acetone extracts exhibited superoxide radical scavenging activity. Ethyl acetate extract scavenged superoxide radicals significantly with low IC_{50} value (Table 1). Superoxide anion plays an important role in the formation of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA (*38,39*).

Lipid peroxidation inhibitory activity

Lipid peroxidation causes destabilization and disintegration of the cell membrane, leading to liver injury, atherosclerosis, kidney damage, aging, and susceptibility to cancer (*40*). Except water extract, all other mango ginger extracts showed potential lipid peroxidation inhibitory activity. The chloroform extract was found to be a

Fig. 1. Phenolic content in mango ginger extracts. The expressed values are means±S.D. of three replicates; those with different letters differ significantly at p<0.05

Table 1. Antioxidant activity of mango ginger extracts

Extracts	IC_{50} values/µg			
	DPPH· scavenging activity	Superoxide scavenging activity	Lipid peroxidation inhibition	Metal chelating activity
hexane	—	—	(94.2±3.3)[b]	(218.1±4.5)[c]
chloroform	—	—	(80.6±4.1)[a]	(142.2±4.6)[a]
ethyl acetate	(77.3±5.8)[b]	(146.5±6.2)[a]	(91.4±2.8)[b]	(168.7±3.6)[b]
acetone	(146.6±3.3)[c]	(288.4±3.2)[b]	(95.3±1.8)[b]	(233.7±5.2)[d]
methanol	(140.5±4.1)[c]	—	(178.2±7.4)[c]	(268.4±5.8)[e]
water	(289.5±6.8)[d]	—	—	—

The expressed values are means±S.D. of three replicates; those with different superscripts differ significantly at p<0.05

potential inhibitor of lipid peroxidation. It is more significant that hexane, chloroform, ethyl acetate and acetone extracts also showed potential activity with low IC_{50} values (Table 1). Even though the phenolic compounds were absent from the chloroform extract, the activity found may be due to the presence of terpenoid compounds, as reported earlier (41,42) along with other compounds. Lipid peroxidation inhibitory activity was mainly attributed to the number of hydroxyl groups and also depends on the solubility, *i.e.* hydrophobicity of the compounds.

Metal chelating activity of mango ginger extracts

Except water extract, all other mango ginger extracts showed metal chelating activity. Ethyl acetate and acetone extracts were found to be potential metal chelators with low IC_{50} values (Table 1). It was reported that the structures containing two or more of the following functional groups: -OH, -SH, -COOH, $-O_3H_2$, C=O, $-NR_2$, -S- and -O- in a favourable structure-function configuration can show metal chelating activity (43,44). It is evident that the mango ginger extracts are potential source of bioactive compounds with some of the above functional groups as reported by Policegoudra *et al.* (24,41,42). Since ferrous ions are the most effective prooxidant in the food system (45), high chelating abilities of mango ginger extracts would be beneficial. Iron can stimulate lipid peroxidation by Fenton reaction, and also accelerate peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl groups (46,47).

Total reducing power of mango ginger extracts

Acetone and ethyl acetate extracts showed high reducing power compared to hexane, chloroform, methanol and water extracts. All the extracts exhibited concentration-dependent activity (Fig. 2). The presence of compounds with -OH groups in the extracts may be responsible for reduction power. The reducing capacity of a compound may serve as a significant indicator of its antioxidant capacity (48).

The antioxidant activity of mango ginger extracts was independent of phenolic content. Polar extracts exhibited potential DPPH radical scavenging activity, superoxide radical scavenging activity, lipid peroxidation inhibitory activity and metal chelating activity, but the non-polar extracts showed potential lipid peroxidation inhibitory activity and metal chelating activity. However, it is known that nonphenolic constituents could also contribute to the antioxidant activity of extracts (49). True to
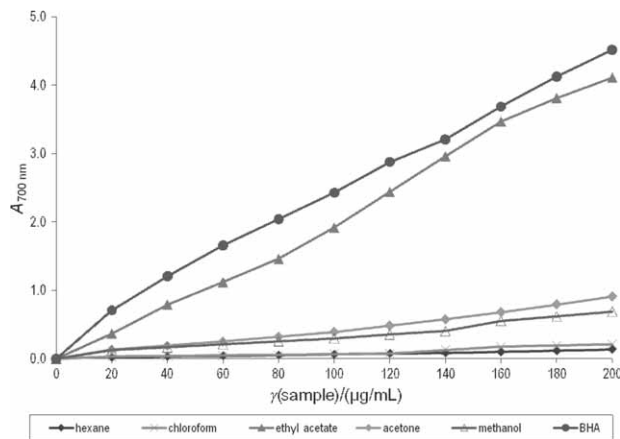

Fig. 2. Total reducing power of mango ginger extracts

this, recently, three new bioactive terpenoid compounds have been isolated and characterized from mango ginger rhizomes, which are highly antibacterial and antioxidant (24,41,42). Hence, antioxidant activity of mango ginger extracts may be due to synergetic effect of phenolic and terpenoid molecules.

Mango ginger extract cytotoxicity

All the extracts of mango ginger showed moderate cytotoxicity (Table 2) towards both normal and cancer cell cultures tested. All extracts were tested for cytotoxicity in the concentration ranging from 10 to 500 µg. The survival studies showed that ethyl acetate extract is more cytotoxic at lower concentration, which may be due to the presence of high phenolic concentration. Hexane and chloroform extracts also showed toxicity towards cancer cell lines with concentration CTC_{50} in the range from 90 to 100 µg, whereas the CTC_{50} of the remaining extracts ranged from 132 to 407 µg. All the extracts showed comparatively more toxicity towards cancer cells in comparison with normal cells. Among the five extracts tested, the ethyl acetate extract showed higher toxicity with CTC_{50} values ranging from 52 to 65 µg, followed by chloroform, hexane, acetone and methanol extracts with lower CTC_{50} values ranging from 90 to 423 µg (Table 2). All the mango ginger extracts also showed cytotoxicity towards normal cell lines with CTC_{50} ranging from 62 to 423 µg. Ethyl acetate extract exhibited cytotoxicity against Vero cell lines with CTC_{50} ranging from 62 to 65 µg. The other extracts also exhibited cyto-
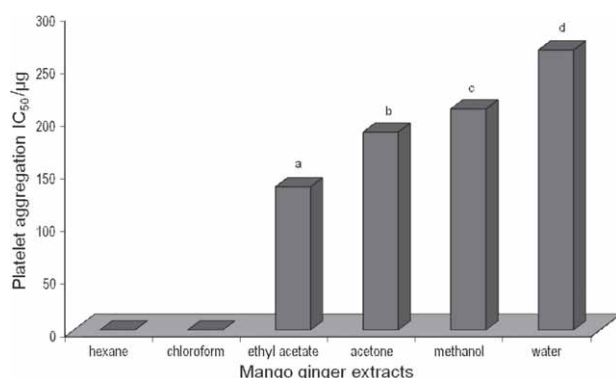
Table 2. Cytotoxicity of mango ginger extracts

| Extracts | CTC_{50} values/µg | | | |
| | Vero cell line | | A-549 cell line | |
	MTT assay	SRB assay	MTT assay	SRB assay
hexane	$(106.3\pm1.8)^b$	$(110.7\pm2.5)^b$	$(95.6\pm2.3)^b$	$(100.3\pm1.4)^b$
chloroform	$(102.3\pm2.3)^b$	$(106.9\pm3.1)^b$	$(90.2\pm1.2)^b$	$(96.7\pm2.3)^b$
ethyl acetate	$(65.1\pm1.6)^a$	$(62.4\pm1.5)^a$	$(52.5\pm1.1)^a$	$(57.5\pm1.7)^a$
acetone	$(137.8\pm1.8)^c$	$(140.5\pm3.2)^c$	$(132.3\pm3.4)^c$	$(138.4\pm2.3)^c$
methanol	$(408.6\pm4.2)^e$	$(423.6\pm3.7)^e$	$(395.2\pm5.2)^d$	$(397.8\pm5.3)^d$
water	$(386.9\pm3.5)^d$	$(393.3\pm3.4)^d$	$(405.2\pm7.3)^e$	$(407.4\pm10.0)^e$

The expressed values are means±S.D. of three replicates; those with different superscripts differ significantly at $p<0.05$

toxicity against normal cell lines with CTC_{50} ranging from 102 to 423 µg. It was observed that except ethyl acetate extract, all mango ginger extracts showed cytotoxicity towards both cell lines at higher concentrations.

Platelet aggregation inhibitory activity

Platelets readily aggregate in response to a variety of endogenous substances and they can initiate thrombus formation, leading to ischemic diseases. In addition, the interactions between platelets and blood vessel walls are important in the development of thrombosis and cardiovascular diseases (*50,51*). Therefore, the inhibition of platelet function represents a promising approach for the prevention of thrombosis. Platelet aggregation inhibitory activity of ethyl acetate and acetone extracts was very high, followed by methanol and water extracts. Hexane and chloroform extracts did not show activity (Fig. 3). The high platelet aggregation inhibitory activity of ethyl

Fig. 3. Platelet aggregation inhibitory activity of mango ginger extracts. The expressed values are means±S.D. of three replicates; those with different letters differ significantly at p<0.05

acetate and acetone extracts (Table 2) may be correlated with high phenolic content. Even though the methanol extract contains high phenolic content, the activity was lower than that of ethyl acetate and acetone extracts.

Conclusion

High antioxidant activity, cytotoxicity and platelet aggregation inhibitory activity of mango ginger extracts can be correlated with their phenolic content and other bioactive constituents. Since these bioactive compounds are known for their health beneficial properties, mango ginger extracts can be used as natural source of phenolic and terpenoid compounds. The antioxidant and platelet aggregation inhibitory activity of mango ginger extracts can be useful for the preparation of culinary and nutraceutical products. However, pharmacological evidence at molecular level is required to establish the possible correlation between medicinal properties of mango ginger constituents and bioactivities of the extracts.

References

1. J.W. Spencer: Essential Issues In Complementary/Alternative Medicine. In: *Complementary/Alternative Medicine: An Evidence-Based Approach*, J.W. Spencer, J.J. Jacobs (Eds.), Mosby, St. Louis, MO, USA (1999) pp. 3–36.

2. F. Fang, S. Sang, K.Y. Chen, A. Gosslan, C.T. Ho, R.T. Rosen, Isolation and identification of cytotoxic compounds from bay leaf (*Laurus nobilis*), *Food Chem. 93* (2005) 497–501.

3. A. Hässig, W.X. Liang, H. Schwabl, K. Stampfli, Flavonoids and tannins: Plant-based antioxidants with vitamin character, *Med. Hypotheses, 52* (1999) 479–481.

4. I.R. Lee, M.Y. Yang, Phenolic compounds from *Duchesnea chrysantha* and their cytotoxic activities in human cancer cell, *Arch. Pharm. Res. 17* (1994) 476–479.

5. C.A. Rice-Evans, J. Miller, G. Paganga, Antioxidant properties of phenolic compounds, *Trends Plant Sci. 2* (1997) 152–159.

6. X. Chen, D.U. Ahn, Antioxidant activities of six natural phenolics against lipid oxidation induced by Fe^{2+} or ultraviolet light, *J. Am. Oil Chem. Soc. 75* (1998) 1717–1721.

7. C.N. Lin, C.M. Lu, Novel antiplatelet constituents from Formosan Moraceous plants, *J. Nat. Prod. 59* (1996) 834–838.

8. I.L. Tsai, W.Y. Lin, C.M. Teng, T. Ishikawa, S.L. Doong, M.W. Huang *et al.*, Coumarins and antiplatelet constituents from the root bark of *Zanthoxylum schinifolium*, *Planta Med. 66* (2000) 618–623.

9. J.H. Cho, D.J. Son, C.H. Lee, H.S. Lee, Y.H. Park, Antiplatelet activity of phenylpropanoids isolated from *Eugenia caryophyllata* leaf oil, *Food Sci. Biotechnol. 13* (2004) 315–317.

10. M.Y. Lim, Y.H. Park, D.J. Son, M.K. Kim, H.S. Lee, Antiplatelet activity of gallic acid and methyl gallate, *Food Sci. Biotechnol 13* (2004) 806–809.

11. N.V. Yanishlieva, E.M. Marinova, Stabilization of edible oils with natural antioxidants, *Eur. J. Lipid Sci. Technol. 103* (2001) 752–767.

12. B. Halliwell, Free radicals, antioxidants, and human disease. Curiosity, cause, or consequence?, *Lancet, 344* (1994) 721–724.

13. D. Dreher, A.F. Junod, Role of oxygen free radicals in cancer development, *Eur. J. Cancer, 32* (1996) 30–38.

14. S.R.Y. Maxwell, Anti-oxidant therapy: Does it have a role in the treatment of human disease?, *Expert Opin. Inv. Drugs, 6* (1997) 211–236.

15. J. Pincemail: Free Radicals and Antioxidants in Human Diseases. In: *Analysis of Free Radicals in Biological Systems*, A.E. Favier, J. Cadet, B. Kalyanaraman, M. Fontecave, J.L. Pierre (Eds.), Birkhäuser Verlag, Basel, Switzerland (1995) pp. 83–98.

16. B. Sasikumar, Genetic resources of *Curcuma*: Diversity, characterization and utilization, *Plant Genet. Resour. 3* (2007) 230–251.

17. *The Wealth of India: Raw Materials, Vol. 2*, CSIR, New Delhi, India (1950) p. 401.

18. *Indian Medicinal Plants, Vol. 4*, K.R. Kirtikar, B.D. Basu (Eds.), Dehra Dun, India (1984) pp. 2422–2423.

19. *Indian Medicinal Plants – A Compendium of 500 Species, Vol. 1*, P.K. Warrier, V.P.K. Nambiar, C. Ramankutty (Eds.), Orient Longman Pvt. Ltd, Chennai, India (1994) p. 106.

20. *Dictionary of Indian Medicinal Plants*, A. Hussain, O.P. Virmani, S.P. Popli, L.N. Misra, M.M. Gupta (Eds.), CIMAP, Lucknow, India (1992) p. 161.

21. M.S. Taga, E.E. Miller, D.E. Pratt, Chia seeds as a source of natural lipid antioxidants, *J. Am. Oil Chem. Soc. 61* (1984) 928–993.

22. M.S. Blois, Antioxidant determinations by the use of a stable free radical, *Nature, 181* (1958) 1199–1200.

23. J.H. Moon, J. Terao, Antioxidant activity of caffeic acid and dihydrocaffeic acid in lard and human low-density lipoprotein, *J. Agric. Food Chem. 46* (1998) 5062–5065.

24. R.S. Policegoudra, K. Rehna, L.J. Rao, S.M. Aradhya, Antimicrobial, antioxidant, cytotoxicity and platelet aggregation inhibitory activity of a novel molecule isolated and

characterized from mango ginger (*Curcuma amada* Roxb.) rhizome, *J. Biosci.* **35** (2010) 231–240.

25. M. Nishikimi, N.A. Rao, K. Yagi, The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen, *Biochem. Biophys. Res. Commun.* **46** (1972) 849–864.

26. P.D. Duh, G.H. Yen, Antioxidative activity of three herbal water extracts, *Food Chem.* **60** (1997) 639–645.

27. E.A. Decker, B. Welch, Role of ferritin as a lipid oxidation catalyst in muscle food, *J. Agric. Food Chem.* **38** (1990) 674–677.

28. T.C. Dinis, V.M. Madeira, L.M. Almeida, Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, *Arch. Biochem. Biophys.* **315** (1994) 161–169.

29. G.C. Yen, H.Y. Chen, Antioxidant activity of various tea extracts in relation to their antimutagenicity, *J. Agric. Food Chem.* **43** (1995) 27–32.

30. F. Denizot, R. Lang, Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability, *J. Immunol. Methods,* **89** (1986) 271–277.

31. P. Skehan, R. Storeng, D. Scudiero, A. Monks, Y. McMahon, D. Vistica, New colorimetric cytotoxic assay for anticancer-drug screening, *J. Natl. Cancer Inst.* **82** (1990) 1107–1112.

32. M.N. Siddaraju, S.M. Dharmesh, Inhibition of gastric H^+, K^+-ATPase and *Helicobacter pylori* growth by phenolic antioxidants of *Curcuma amada*, *J. Agric. Food Chem.* **55** (2007) 7377–7386.

33. M. López-Vélez, F. Martinez-Martinez, C. Del Valle-Ribes, The study of phenolic compounds as natural antioxidants in wine, *Crit. Rev. Food Sci. Nutr.* **43** (2003) 233–244.

34. C.A. Rice-Evans, N.J. Miller, P.G. Bolwell, P.M. Bramley, J.B. Pridham, The relative antioxidant activities of plant-derived polyphenolic flavonoids, *Free Radic. Res.* **22** (1995) 375–383.

35. T. Hatano, R. Edamatsu, M. Hiramatsu, A. Mori, Y. Fujita, E. Yasuhara *et al.*, Effect of interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical, *Chem. Pharm. Bull.* **37** (1989) 2016–2021.

36. C.W. Chen, C.T. Ho, Antioxidant properties of polyphenols extracted from green and black teas, *J. Food Lipids,* **2** (1995) 35–46.

37. N. Nenadis, H.Y. Zhang, M.Z. Tsimidou, Structure-antioxidant activity relationship of ferulic acid derivatives: Effect of carbon side chain characteristic groups, *J. Agric. Food Chem.* **51** (2003) 1874–1879.

38. B. Halliwell, J.M. Gutteridge: *Free Radicals in Biology and Medicine*, Clarendon Press, Oxford, UK (1989) pp. 23–30.

39. P.G. Pietta, Flavonoids as antioxidants, *J. Nat. Prod.* **63** (2000) 1035–1042.

40. C. Rice-Evans, R. Burdon, Free radical-lipid interactions and their pathological consequences, *Prog. Lipid Res.* **32** (1993) 71–110.

41. R.S. Policegoudra, S. Divakar, S.M. Aradhya, Identification of difurocumenonol, a new antimicrobial compound from mango ginger (*Curcuma amada* Roxb.) rhizome, *J. Appl. Microbiol.* **102** (2007) 1596–1602.

42. R.S. Policegoudra, K. Abiraj, D. Channe Gowda, S.M. Aradhya, Isolation and characterization of antioxidant and antibacterial compound from mango ginger (*Curcuma amada* Roxb.) rhizome, *J. Chromatogr. B,* **852** (2007) 40–48.

43. R.C. Lindsay, A.L. Branen, P.M. Davidson, S. Salminen: Food Additives. In: *Food Chemistry*, O.R. Fennema (Ed.), Marcel Dekker Inc., New York, NY, USA (1996) pp. 778–780.

44. Y.V. Yuan, D.E. Bone, M.F. Carrington, Antioxidant activity of dulse (*Palmaria palmata*) extract evaluated *in vitro*, *Food Chem.* **91** (2005) 485–494.

45. R. Yamaguchi, M.A. Tatsumi, K. Kato, U. Yoshimitsu, Effect of metal salts and fructose on the autoxidation of methyl linoleate in emulsions, *Agric. Biol. Chem.* **52** (1988) 849–850.

46. B. Halliwell, Reactive oxygen species in living systems: Source, biochemistry, and role in human disease, *Am. J. Med.* **91** (1991) 14–22.

47. I. Gulcin, M.E. Buyukokuroglu, O.I. Kufrevioglu, Metal chelating and hydrogen peroxide scavenging effects of melatonin, *J. Pineal Res.* **34** (2003) 278–281.

48. S. Meir, J. Kanner, B. Akiri, S. Philosoph-Hadas, Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves, *J. Agric. Food Chem.* **43** (1995) 1813–1817.

49. N. Mariko, A. Hassimotto, M.I. Genovese, F.M. Lajolo, Antioxidant activity of dietary fruits, vegetables, and commercial frozen pulps, *J. Agric. Food Chem.* **53** (2005) 2928–2935.

50. J.L. Dinerman, J.L. Mehta, Endothelial, platelet and leukocyte interactions in ischemic heart disease: Insights into potential mechanisms and their clinical relevance, *J. Am. Coll. Cardiol.* **16** (1990) 207–222.

51. J. Hirsh, Hyperactive platelets and complications of coronary artery disease, *New Eng. J. Med.* **316** (1987) 1543–1544.