Chapter 1

Functional Properties of Mango Ginger Extracts

Research is to see what everybody else has seen, And to think what nobody else has thought
— Albert Szent-Gyorgyi
Ayurveda, Unani and other traditional medicines have gained a momentum due to their totalitarian approach. Potential application of mango ginger rhizome extracts in Ayurveda, Unani and other traditional medicines to reduce oxidative stress and as a source of natural antimicrobial along with other health benefits is well documented. Further, infectious and degenerative diseases are becoming more persistence due increasing resistance of microorganisms to the existing synthetic drugs. In this contest, natural, multifunctional, stable, non-toxic and natural bioactive compounds from plants may prefer panacea for disease causing microbes. Fruits, vegetables, spices, tubers and roots are rich in phytochemicals that provide protection against various chronic and degenerative diseases and also impart other health benefits [Wildman, 2001].

A systematic review of literature on research work on mango ginger has revealed that, earlier reports have concentrated only on volatile constituents and water extract of mango ginger rhizome. Surprisingly, there are no reports on isolation and characterization of bioactive compounds. Water extract was not encouraged due to its inadequacy to extract all types of bioactive compounds that are soluble in lipids or sparingly soluble in water [Zhang and Lewis, 1997]. Hence, we have carried out sequential extraction of dried mango ginger powder with the solvents of increasing polarity. All the extracts were tested for an array of functional properties like- antibacterial activity, antioxidant activity, platelet-aggregation inhibitory activity and cytotoxicity. The details of the work carried out are presented in this chapter.
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 hrs and powdered to 100-120 meshes in an apex grinder [Apex Constructions, London].

Preparation of extracts

Sequential extraction was carried out using solvents of different polarity [from non-polar to polar]. Sequential extraction was employed to resolve the compounds of different polarity effectively and completely. About 100 g of dry mango ginger powder was sequentially extracted using n-hexane, followed by chloroform, ethyl acetate, acetone, methanol and water at room temperature [25±2°C], at normal atmospheric pressure, by shaking at 100 rpm for 48 hrs. Each extract was filtered and concentrated by using rotary evaporator [Buchi Rotavapor R-124, Switzerland]. The concentrated extracts were freeze-dried and stored in refrigerator till used.

Determination of Phenolics

Total phenolic content was determined with the modified method of Taga, et al., [1984]. In brief, 100 µl of test sample was mixed with 2 ml of 2 % sodium carbonate solution. After 3 min, 100 µl of 50 % of Folin-Ciocalteau’s phenol reagent was added to the mixture. After 30 min of incubation at room temperature, absorbance was measured at 750 nm against a blank. Total phenolic content was calculated on the basis of the calibration curve of gallic acid and expressed as gallic acid equivalents.

ANTIBACTERIAL ACTIVITY

Bacterial strains and Inoculum preparation

The antibacterial activity was tested against Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Enterobacter aerogenes, Proteus mirabilis, Yersinia enterocolitica, Micrococcus luteus, Staphylococcus aureus, Enterococcus fæcalis, Bacillus subtilis, Bacillus cereus and Listeria monocytogenes. The above bacterial strains isolated from clinical samples were obtained from the Department of Microbiology,
Mysore Medical College, Mysore, India. Their cultural characteristics and morphological features were reconfirmed and also subjected to standard biochemical tests [Krieg and Holt 1984; Sneath et al., 1986] before experimentation. The test organisms were maintained on nutrient agar slants.

**Agar-well diffusion method**

*In vitro* antibacterial activity was determined by agar-well diffusion method [Mukherjee et al., 1995]. The overnight bacterial culture was centrifuged at 8000 rpm for 10 minutes at 4°C. The supernatant was discarded and bacterial cells were resuspended in the saline to make suspension $10^5$ CFU ml$^{-1}$ and used for the assay. The plating was carried out by transferring bacterial suspension $[10^5$ CFU ml$^{-1}]$ to sterile Petri plate and mixed with molten Nutrient agar medium [Hi-Media Laboratories Limited, Mumbai, India] and allowed to solidify. About 75 μl of the sample [2 mg ml$^{-1}$] was placed in the wells and allowed to diffuse for 2 h. Plates were incubated at 37°C for 48 h and the activity was determined by measuring the diameter of inhibition zones. Solvent control and amoxicillin [Galpa Lab. Mumbai, India] were also maintained. The assay was carried out in triplicate.

**Minimum inhibitory concentration [MIC]**

The minimum inhibitory concentration was determined according to the method described by Jones et al., [1985]. Different concentrations [20 ppm to 300 ppm] of hexane, chloroform, ethyl acetate, acetone and methanol extracts and 100 μl of the bacterial suspension $[10^5$ CFU ml$^{-1}]$ was placed aseptically in 10 ml of nutrient broth separately and incubated for 24 h at 37°C. The growth was observed both visually and by measuring O.D. at 600 nm at regular intervals followed by pour plating as described above. The lowest concentration of test sample showing no visible growth was recorded as the minimum inhibitory concentration. Triplicate sets of tubes were maintained for each concentration of test sample.

**Determination of minimum bactericidal concentration [MBC]**

Minimum bactericidal concentration [MIC] was determined according to the method of Smith-Palmer et al., [1998]. Test tubes containing nutrient broth with different concentrations of hexane, chloroform, ethyl acetate, acetone and methanol extracts were
inoculated with 100 µl of the bacterial suspension \([10^5 \text{ CFU ml}^{-1}]\). Inoculated tubes were incubated for 24 h at 37°C and growth was observed both visually and by measuring O.D. at 600 nm. About 100 µl from the tubes not showing growths were plated on nutrient agar as described above. Minimum bactericidal concentration is the concentration at which bacteria failed to grow in nutrient broth and nutrient agar inoculated with 100 µl suspension. Triplicate sets of tubes were maintained for each concentration of test sample.

**ANTIOXIDANT ACTIVITY**

**DPPH free radical scavenging activity**

DPPH [1, 1-diphenyl-2-picrylhydrazyl] radical scavenging activity was determined according to the method described earlier [Blois, 1958; Bondet et al., 1997; Moon and Terao, 1998]. The test samples [10-100 µl] were mixed with 0.8 ml of Tris-HCl buffer [pH 7.4] to which 1 ml of DPPH [500 µM in ethanol] was added. The mixture was shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm in a UV-Visible Spectrophotometer [UV-160A, Shimadzu co. Japan]. The radical scavenging activity was measured as a decrease in the absorbance of DPPH. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical scavenging potential was expressed as EC$_{50}$ value, which represents the sample concentration at which 50 % of the DPPH radicals scavenged.

**Superoxide radical scavenging activity**

The superoxide scavenging ability was assessed according to the method of Nishikimi, et al., [1972] with slight modifications. The reaction mixture contained NBT [0.1 mM] and NADH [0.1 mM] with or without sample to be assayed in a total volume of 1 ml of Tris-HCl buffer [0.02 M, pH 8.3]. The reaction was started by adding PMS [10 µM] to the mixture, and change in the absorbance was recorded at 560 nm every 30 seconds for 2 min. The percent inhibition was calculated against a control without test sample. Radical scavenging potential was expressed as EC$_{50}$ value, which represents the sample concentration at which 50 % of the radicals scavenged.
**Lipid peroxidation inhibitory activity**

Lipid peroxidation inhibitory activity was determined according to the method described earlier [Duh and Yen, 1997]. In brief, lecithin [3 mg/ ml phosphate buffer, pH 7.4] was sonicated in dr. Hielscher GmbH, UP 50H ultraschallprozessor [DrHielscher GmbH, Teltow, Berlin, Germany]. The test samples [100 µl] were added to 1ml of liposome mixture, control was without test sample. Lipid peroxidation was induced by adding 10 µl FeCl₃ [400 mM] and 10 µl L-ascorbic acid [400 mM]. After incubation for 1 hour at 37°C, the reaction was stopped by adding 2 ml of 0.25 N HCl containing 15 % TCA and 0.375 % TBA and the reaction mixture was boiled for 15 min. then cooled, centrifuged and absorbance of the supernatant was measured at 532 nm. Inhibitory activity was expressed as EC₅₀ value, which is sample concentration inhibited 50 % of lipid peroxidation.

**Metal chelating activity**

The chelating of ferrous ions by the test sample was estimated by the method described earlier [Decker and Welch, 1990; Dinis et al., 1994]. Briefly, the test samples at different concentrations were added to a solution of 2mM FeCl₂ [0.05 ml]. The reaction was initiated by the addition of 5mM ferrozine [0.2 ml] and the mixture was vigorously shaken and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the mixture was read at 562nm against a blank. EDTA was used as positive control. Results were expressed as EC₅₀ value, which represents the sample concentration at which 50 % of metal chelation occurred.

**Total reducing power**

The reducing power was quantified by the method described earlier by Yen and Chen [1995] with minor modifications. Reaction mixture, containing test samples at different concentrations [10-100 µl] in phosphate buffer [0.2 M, pH 6.6], was incubated with potassium ferricyanide [1 % w/v] at 50°C for 20 min. The reaction was terminated by the addition of TCA solution [10 % w/v] and the mixture was centrifuged at 3000 rpm for 20 min. The supernatant was mixed with distilled water and ferric chloride [0.1 % w/v] solution and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.
PLATELET-AGGREGATION INHIBITORY ACTIVITY

Platelet preparation

Blood samples were taken from healthy volunteers who assured not to have taken any drugs during the 2 weeks prior to the blood sampling. Blood was collected into buffered sodium citrate [3.8 % w/v] pH 6.5 as the anticoagulant at a ratio of 9:1 v/v and used within 3 hr of collection. Platelet-rich plasma [PRP] was obtained by centrifugation of the citrated blood at 1100 rpm for 20 min. the residual blood was again centrifuged at 2500 rpm for 20 min to obtain the homologous Platelet Poor Plasma [PPP]. Platelet count was adjusted to 1.6 x 10^7 platelets per μl of PRP.

Platelet-aggregation assay

Aggregation was measured turbidimetrically at 37°C with constant stirring at 1000 rpm in a Chronolog Dual Channel Aggregometer. About 0.45 ml of PRP was kept stirred at 1200 rpm at 37°C, and aggregation was induced by collagen [10 μM] and. The change in turbidity was recorded with reference toPPP using an omnisccribe recorder for at least 5 min. The slope was calculated and it was used as control.

Similarly, 100-500 μM of the mango ginger extracts and isolated bioactive compounds were added to PRP, incubated for five min after which collagen [10 μM], was added. Platelet aggregation was recorded using an omnisccribe recorder for 5 min. The slope was calculated. The difference in the slope between the control and the treated was expressed as percent inhibition of platelet aggregation by mango ginger extracts.

CYTOTOXICITY OF MANGO GINGER EXTRACTS

Chemicals

Sulforhodamine B [SRB], 3-[4, 5-dimethyl thiazol-2-yl]-5-diphenyl tetrazolium bromide [MTT], New Born Calf Serum [NBCS] were obtained from Sigma Aldrich Co, St Louis, USA., Phosphate Buffered Saline [PBS], Dulbecco’s Modified Eagle’s medium [DMEM] and antibiotics from Hi-Media Laboratories Ltd., Mumbai., Trichloro acetic acid [TCA] and tris buffer from SD fine chemicals Pvt. Ltd., Boisar, India., 25 cm² and 75 cm² tissue culture flasks, 96 well microtitre plates were procured from Tarson India Pvt. Ltd., Kolkata, India, DMSO, glacial acetic acid and propanol from E-Merck Ltd., Mumbai, India.


**Preparation of test solutions**

For cytotoxicity studies, each extract was weighed separately, dissolved in distilled dimethyl sulfoxide [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 and sterilized by filtration and stored at -20°C till use. Serial two fold dilution of the extracts was prepared from the stock solution to obtain lower concentrations.

**Cell lines and culture medium**

Vero [normal African Green Monkey Kidney] cell culture was procured from National Centre for Cell Sciences [NCCS], Pune, India., 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 % CO₂ at 37°C until 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 cm² flat bottles and all experiments were carried out in either 96 well microtitre plates.

**Determination of mitochondrial synthesis by MTT assay**

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The cleavage of MTT to a blue formazan derivative by living cells is clearly a very effective principle on which the assay is based. The principle involved is the cleavage of tetrazolium salt 3-[4, 5 dimethyl thiazole-2-yl]-2,5-diphenyl tetrazolium bromide [MTT] into a blue coloured product [formazan] by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used [Francis and Rita, 1986].
Procedure

The monolayer cell culture was trypsinized and cell count adjusted to 1.0 \times 10^5 cells/ml using DMEM medium containing 10% NBCS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension [approximately 10,000 cells] was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 \mu l of different extract concentrations were added to the cells in microtitre plates. The plates were then incubated at 37\degree C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h. After 72 h, the extract solutions in the wells were discarded and 50 \mu l of MTT in DMEM-PR [Dulbecco’s Modified Eagle’s medium without phenol red, 2 mg/ml] was added to each well. The plates were gently shaken and incubated for 3 h at 37\degree C in 5% CO_2 atmosphere. The supernatant was removed and 50 \mu l of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and \text{CTC}_{50} [concentration of drug or test extract needed to inhibit cell growth by 50\%] values were generated from the dose-response curves for each cell line. The pattern of all the cell lines as a group is used to rank compounds as toxic or non-toxic.

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\% \text{ Growth Inhibition} = 100 - \left( \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \right) \times 100
\]

Determination of total cell protein content by Sulforhodamine B [SRB] assay

SRB is a bright pink aminoxanthine dye with two sulfonic groups. Under mild acidic conditions, SRB binds to protein basic amino acid residues in trichloro acetic acid [TCA] fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of at least two orders of magnitude. Colour development in SRB assay is rapid, stable and visible. The developed colour can be measured over a broad range of visible wavelength in either a spectrophotometer or a 96 well plate reader. When TCA-fixed and SRB stained samples are air-dried, they can be stored indefinitely without deterioration [Philip et al., 1990].
Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 × 10^5 cells/ml using DMEM medium containing 10 % NBCS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension [approximately 10,000 cells] was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 μl of different extract concentrations were added to the cells in microtitre plates. The plates were then incubated at 37°C for 3 days in 5 % CO₂ atmosphere, microscopic examination was carried out and observations were recorded every 24 h. After 72 h, 25 μl of 50 % trichloro acetic acid was added to the wells gently in such a way that it forms a thin layer over the extract to form an over all concentration of 10 %. The plates were incubated at 4°C for 1 h. The plates were flicked and washed five times with water to remove traces of medium, extract and serum, and air-dried. They were stained with SRB [0.4 % prepared in 1 % acetic acid] for 30 min. The unbound dye was then removed by rapidly washing four times with 1 % acetic acid. The plates were then air-dried. Tris base [10 mM, 100 μl] was then added to the wells to solubilise the dye. The plates were shaken vigorously for 5 min. The absorbance was measured using microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the same formula used for MTT assay. CTC₅₀ values were calculated.
**Results and Discussion**

Sequential extraction of 100 g of mango ginger powder using hexane, chloroform, ethyl acetate, acetone and methanol yielded 11 g, 8 g, 0.9 g, 0.8 g and 10 g of extract respectively [Fig. 1.1]. This kind of extraction method will be helpful to extract wide range of [both non-polar and polar] bioactive molecules in a plant material. Each extract was tested for the antibacterial activity, antioxidant activities, platelet-aggregation inhibitory activity and cytotoxicity.

**Solvent V/S active components**

The extraction procedure depends upon the type of bioactive component to be extracted. Polar components such as phenolic acids and glycosides of many flavonoids are generally extracted using water, alcohols or a mixture of water and alcohols. For flavonoids and most carotenoids, non-polar solvents are used [Tsao and Deng, 2004]. It has been indicated that methanol is the best solvent for extraction of polyphenols, lactones, phenones, quassinoids, flavones, saponons and some terpenoids. It has also been indicated that acetone/water mixtures are more useful for extracting polyphenols from proteic matrices, since they appear to degrade the polyphenols-protein complexes [Cowan, 1999; Suhaj, 2006].
**Phenolic content in mango ginger extracts**

Among five mango ginger extracts, the methanol extract showed high phenolic content [178±7 mg] followed by acetone [128±5 mg], ethyl acetate [112±7 mg] extract [Fig. 1.2]. The phenolic compounds were not detected in hexane, chloroform extracts. The concentrations of phenolics in the extracts were expressed as mg /100 g.

Plant phenolics present in the fruit and vegetables have received considerable attention because of their potential antioxidant activity [Lopez-Velez, *et al.*, 2003]. Phenolic compounds are the major contributors of antioxidant activity in vegetable juices [Gardner, *et al.*, 2000]. Phenolic compounds are effective hydrogen donors, which make them good antioxidant [Rice-Evans, *et al.*, 1995].

The antioxidant activity 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 non-phenolic antioxidants could also contribute to the antioxidant activity of extracts [Mariko, *et al.*, 2005]. Antioxidant-rich plant extracts serve as sources of nutraceuticals that alleviate the oxidative stress and therefore prevent or slow down the degenerative diseases [Ames *et al.*, 1993; Cao *et al.*, 1996; Kitts *et al.*, 2000; Fu and Ji, 2003]. The broad range of antioxidant activity of the mango ginger extracts indicates the potential of the rhizomes as a source of natural antioxidants or nutraceuticals with potential application to reduce oxidative stress with consequent health benefits.

*Fig. 1.2: Phenolic content in mango ginger extracts*

*Values expressed are means ± SD of three replicates.
Values with different letters (a, b and c) differ significantly at *P* < 0.05*
Antibacterial activity of mango ginger extracts

Among the five extracts of mango ginger, chloroform extract showed antibacterial activity against seven out of 13 bacteria screened viz. B. cereus, B. subtilis, M. luteus, S. aureus, L. monocytogenes, E. fecalis and S. typhi. Hexane extract, similar to chloroform extract, showed inhibition to all the bacteria except S. typhi. Ethyl acetate extract showed inhibitory effect as shown by chloroform extract, except S. typhi and L. monocytogenes. Acetone extract inhibited M. luteus, L. monocytogenes while methanol extract showed inhibition only against E. faecalis.

The chloroform extract of mango ginger exhibited highest antibacterial activity against wide range of bacteria, when compared to other solvent extracts [Table 1.1]. However, E. coli, E. aerogenes, K. pneumoniae, P. aeruginosa, P. mirabilis and Y. enterocolitica were not inhibited by any of the solvent extracts of mango ginger.

Different solvent extracts of mango ginger showed MIC values ranged from 60-220 ppm [Table 1.1]. The high antibacterial activity i.e. low MIC for wide range of bacteria was exhibited by chloroform extract. Chloroform extract was very effective against B. subtilis, B. cereus with MIC of 60 ppm and also inhibited the growth of M. luteus and S. aureus at 80 ppm, while S. typhi, E. fecalis and L. monocytogenes were completely inhibited at 180, 140 and 100 ppm respectively. In contrast, lack of outer polysaccharides layer in Gram-positive bacteria may be responsible for more permeable to

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC (in ppm) * for mango ginger extracts</th>
<th>Hexane extract</th>
<th>Chloroform extract</th>
<th>Ethyl acetate extract</th>
<th>Acetone extract</th>
<th>Methanol extract</th>
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<td>P. aeruginosa</td>
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<td>180&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>80&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>200&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>E. fecalis</td>
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<td>220&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>--</td>
<td>140&lt;sup&gt;c&lt;/sup&gt;</td>
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* Each value represents mean of three different observations.
Mean values with different superscripts ("a") differ significantly at P<0.05
amphiphatic compounds [Cowan 1999]. However, *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis* and *Y. enterocolitica*, were not inhibited by the entire range of solvent extracts of mango ginger [Table 1.1]. High resistance of Gram-negative bacteria to mango ginger extracts may be due to the presence of their outer layer composed of lipopolysaccharides.

The results indicated differential activity between polar and non-polar solvent extracts of mango ginger. It appeared that with the increase in polarity of the solvent of mango ginger extracts, there was a decrease in potential of antibacterial activity and decrease in range. According to Cowan [1999], polar extracts are less effective against microbes than non-polar extracts. It may be due to the presence of polysaccharides, polypeptides and lectins that are more effective as inhibitors of pathogen adsorption and would not be identified in the screening techniques commonly used.

**Antioxidant activity of mango ginger extracts**

To understand nature and function of antioxidant activity of the extracts, various assays like DPPH radical scavenging activity, superoxide radical scavenging activity, lipid peroxidation inhibitory activity, metal chelating activity and total reducing power were tested. BHA was used as a standard for antioxidant assay.

**DPPH radical scavenging activity**

Except hexane and chloroform extracts all mango ginger extracts showed DPPH scavenging activity. Ethyl acetate extract exhibited potential radical scavenging activity with very low IC₅₀ [77±5]. While acetone and methanol extracts showed less activity with high IC₅₀ values of 146 and 140 respectively [Table 1.2]. The high antioxidant activity of ethyl acetate extract may be due to the cumulative effect of its potential antioxidant compounds along with phenolics. Phenolics are very important bioactive constituents of mango ginger rhizome and tuber crops which are known for their radical scavenging ability due to their hydroxyl groups [Hatano, *et al.*, 1980]. Even though the concentration of phenolic compound is less in ethyl extract compared to other extracts of mango ginger rhizome [Fig. 1.2], it may be having other potential radical scavenging bioactive compounds. DPPH radical scavenging activity may be attributed to the presence of hydrogen-donating ability of -OH and -CH₃ groups in extracts/ compounds [Chen and Ho, 1995; Nikolaos, *et al.*, 2003].
**Superoxide radical scavenging activity**

Among five 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 of 77±5 µg [Table 1.2]. 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 [Dahl and Richardson, 1978; Halliwell and Gutteridge, 1989; Pietta, 2000].

**Table 1.2: Antioxidant activity of mango ginger extracts**

<table>
<thead>
<tr>
<th>EXTRACTS</th>
<th>DPPH scavenging activity</th>
<th>Superoxide scavenging activity</th>
<th>Lipid-peroxidation inhibitory</th>
<th>Metal chelating activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>--</td>
<td>--</td>
<td>94 ± 3$^c$</td>
<td>218 ± 4$^c$</td>
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<td>Chloroform</td>
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<td>80 ± 4$^a$</td>
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<td>Ethyl acetate</td>
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<td>146 ± 6$^a$</td>
<td>91 ± 2$^b$</td>
<td>168 ± 3$^b$</td>
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<tr>
<td>Acetone</td>
<td>146± 3$^b$</td>
<td>288 ± 3$^b$</td>
<td>95 ± 1$^c$</td>
<td>233 ± 5$^d$</td>
</tr>
<tr>
<td>Methanol</td>
<td>140± 4$^b$</td>
<td>--</td>
<td>178 ± 7$^d$</td>
<td>268 ± 5$^e$</td>
</tr>
</tbody>
</table>

* Values expressed are means ± SD of three replicates.
* Values with different superscripts ($^a$, $^b$ and $^c$) differ significantly at P<0.05

**Lipid peroxidation inhibitory (LPI) activity**

All the mango ginger extracts showed potential lipid peroxidation inhibitory activity. The chloroform extract was found to be more potential inhibitor of lipid peroxidation with an IC$_{50}$ value of 80±4 µg. Hexane, ethyl acetate and acetone extracts showed potential LPI activity with IC$_{50}$ value ranged from 91-95 µg [Table 1.2]. Even though the phenolic compounds were absent in chloroform extract [Fig. 1.2] the LPI activity may be due to the presence of terpenoids and other non-polar compounds. Lipid peroxidation inhibitory activity was mainly depends upon the solubility, hydrophobicity of the compounds present in the respective extracts. Lipid peroxidation causes destabilization and disintegration of the cell membrane, leading to liver injury, atherosclerosis, kidney damage, aging, and susceptibility to cancer [Rice-Evans and Burdon, 1993].
Metal chelating activity

All mango ginger extracts showed metal chelating activity [Table 1.2]. Ethyl acetate and acetone extracts were found to be potential metal chelator with low IC\textsubscript{50} value of 142 ± 4 and 168 ± 3 µg respectively. It was reported that the structures containing two or more of the following functional groups: -OH, -SH, -COOH, -O\textsubscript{2}H\textsubscript{2}, C=O, -NR\textsubscript{2}, -S- and -O- in a favorable structure-function configuration can show metal chelating activity [Lindsay, 1996; Yuan, et al., 2005]. Hence, Mango ginger extracts may be potential source of compounds having above functional groups. Since ferrous ions are the most effective prooxidant in the food system [Yamaguchi, et al., 1988] the high chelating abilities of mango ginger extracts would be beneficial. Iron can stimulate lipid peroxidation by Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy [Halliwell, 1991; Gulcin, et al., 2003].

Total reducing power

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. 1.3]. The presence of compounds with -OH groups in the extracts may be responsible for reduction power. The reducing capacity of extracts may serve as a significant indicator of its antioxidant capacity [Meir, et al., 1995].

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 [Ross, 1978; Hirsh, 1987; Dinerman and Mehta,
1990]. Therefore, the inhibition of platelet function represents a promising approach for the prevention of thrombosis.

Platelet aggregation inhibitory activity of ethyl acetate extract [IC$_{50}$ = 136±6 µg] and acetone extract [IC$_{50}$ = 188±7 µg] was very high followed by methanol [IC$_{50}$ = 210 µg extract]. Hexane and chloroform extracts did not show activity [Fig. 1.4]. The high platelet-aggregation inhibitory activity of ethyl acetate, acetone and methanol extracts appears to be correlated with high phenolic content and concentration dependent [Fig. 1.4].

**Cytotoxicity of mango ginger extracts**

All the five extracts of mango ginger showed moderate cytotoxicity [Table 1.3] towards both normal and cancer cell cultures tested. All the extracts showed comparatively more toxicity towards cancer cells when compared to normal cells which is a good indicator of anticancer property of extracts. Among the five extracts tested, the ethyl acetate extract showed more toxicity with CTC$_{50}$ values ranging between 52 to 65 µg/ml concentration, followed by chloroform [between 90-106 µg/ml], hexane [95-110 µg/ml], acetone [132-140 µg/ml] and methanol [395-423 µg/ml] concentration. The cytotoxicity results of different extracts of mango ginger indicate that the extracts are not toxic towards the cells.

![Fig. 1.4: Platelet-aggregation inhibitory activity of mango ginger extracts](image)

*Fig. 1.4: Platelet-aggregation inhibitory activity of mango ginger extracts*

*Values expressed are means of three replicates.
Values with different letters (a, b and c) differ significantly at $P<0.05$

### Table 1.3: Cytotoxicity of mango ginger extracts

<table>
<thead>
<tr>
<th>EXTRACTS</th>
<th>CTC$_{50}$ [µg/ml] *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MTT</td>
</tr>
<tr>
<td>Hexane</td>
<td>106±1 b</td>
</tr>
<tr>
<td>Chloroform</td>
<td>102±2 b</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>65±1 a</td>
</tr>
<tr>
<td>Acetone</td>
<td>137±1 c</td>
</tr>
<tr>
<td>Methanol</td>
<td>408±4 d</td>
</tr>
</tbody>
</table>

*Values expressed are means ± SD of three replicates
Values with different superscripts [a, b and c] differ significantly at $P<0.05$
Conclusion

In the present investigation, screening of mango ginger extracts for different functional properties has revealed that, they are potential inhibitors of bacteria and also scavengers of free radicals, metal chelator and lipid peroxidation-inhibitors. Mango ginger extracts also showed platelet-aggregation inhibitory activity and cytotoxicity properties. The highest antibacterial and antioxidant activities exhibited by mango ginger chloroform extract prompted us for further purification and characterization of bioactive compounds from this extract.