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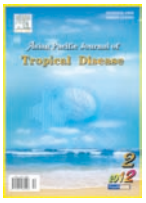

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Antimutagenic and antibacterial activities of *Peltophorum ferrugineum* flower extracts

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ABSTRACT

Objective: To study the antibacterial and antimutagenic properties of the *Peltophorum ferrugineum* flower extracts. **Methods:** Dried flowers of *P. ferrugineum* were extracted successively with hexane, ethyl acetate, acetone and methanol, and the total phenolic content of extracts were determined spectrophotometrically at 760 nm after reaction with the Folin-Ciocalteu reagent. The extracts were tested against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Yersinia enterocolitica* by agar dilution method. The *antimutagenicity* of extracts was studied using the tester strains of *Salmonella typhimurium* by the standard plate incorporation test. The effect of extracts on nucleic acid leakage (spectrophotometrically at 260 nm), bacterial respiration (total dissolved oxygen) and bacterial cell wall (Scanning Electron Microscopy) were also determined. **Results:** The total phenolic content of extracts was in the order of methanol > acetone > hexane > ethyl acetate. All the extracts showed antibacterial activity with minimum inhibitory concentration (MIC) ranging from 0.1 to 1.25%. However, higher activity was found with acetone and methanol extracts. The acetone and methanol extracts showed strong antimutagenic activity against sodium azide and methyl methane sulfonate induced mutation in *Salmonella* tester strains. The antibacterial action of extracts was probably due to the ability of these extracts to cause the disintegration of cell wall, leakage of genetic material and inhibition of respiration. **Conclusions:** It can be concluded that the acetone and methanol extracts of *P. ferrugineum* possess antibacterial and antimutagenic activities, and can find application as food preservatives and nutraceuticals.

1. Introduction

The identification and evaluation of natural products are important challenges for the control of pathogens to assure consumers a safe, wholesome and nutritious food supply. Due to the negative consumer perceptions of artificial preservatives, attention is shifting towards alternatives that the consumers perceive as natural. Therefore, plant extracts including their essential oils are being explored for their bioactive properties. Further, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has increased interest in the antimicrobial activity of plants.

Crude extracts and purified compounds from various plants including *Peltophorum pterocarpum*, *P. africana* and *P. vogelianum* have shown various biological activities such as antimicrobial, antiinflammatory and cytotoxic [1-4]. As per the literature survey, there is no report about the antimutagenic and antibacterial properties of the flowers of *P. ferrugineum*. In the present study, we have studied the antibacterial and antimutagenic properties of the *P. ferrugineum* flower extracts for their possible use as food biopreservatives and nutraceuticals.

2. Materials and methods

2.1. Plant materials and chemicals

The flowers of *Peltophorum ferrugineum* were collected from the campus of CFTRI, Mysore, India. The plant material was identified by Mr. A. S. Chauhan, Scientist, Fruit and Vegetable Technology Department, CFTRI, Mysore, and a

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specimen voucher was deposited in the Fruit and Vegetable Technology departmental herbarium (FVT DH No. CMP-PLT-FRG-5A & 5B/ 2010). All the solvents and chemicals used were of AR grade.

2.2. Extraction

Dried flowers of *P. ferrugineum* were powdered and successively extracted with hexane, ethyl acetate, acetone and methanol using a Soxhlet extractor for 8 h each at 60 ℃. The extracts were filtered through Whatman filter paper No. 1 and concentrated under vacuum to obtain crude viscous extracts. Further, the extracts were dried in vacuum at 45 ℃ for complete removal of solvents. The solvent free extracts were dissolved in propylene glycol (PG) and used for testing the biological activities.

2.3. Determination of total phenolics

The total phenolic content of the extracts from the flowers of *P. ferrugineum* was determined spectrophotometrically at 760 nm (Spectronic 20 Genesys visible spectrophotometer, Spectronic Instruments Inc., NY, USA) after reaction with the Folin-Ciocalteu reagent [5]. The results were expressed as gallic acid equivalent.

2.4. Antibacterial activity

The flower extracts of *P. ferrugineum* were tested against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Yersinia enterocolitica* essentially by the method of Negi et al [6]. One hundred μl of overnight grown bacterium diluted to 10^3 cfu/ml was inoculated into the flask containing 20 ml nutrient agar and different concentrations of flower extracts, and the contents were poured into the sterilized Petri plates. The plates were observed for bacterial growth after overnight incubation at 37 ℃ and minimum inhibitory concentration (MIC) was defined as the lowest concentration of the compound capable of inhibiting the complete growth of bacteria.

2.5. *Antimutagenicity* assay

The *antimutagenicity* of acetone and methanol extracts of *P. ferrugineum* flowers was studied using the tester strains of *Salmonella typhimurium* by the standard plate incorporation test [7]. The test samples along with 0.1 ml of 10 h old culture of strains of *Salmonella typhimurium* in molten soft agar (2 ml) containing 0.2 ml histidine-biotin solution were plated onto minimal glucose agar plates and incubated at 37 ℃ for 48 h. The inhibition of mutagenicity was calculated according to Eq. (1).

$$I = (1 - T/M) \times 100 \quad (1)$$

where, I is the % Inhibition, T is the number of revertants per plate in presence of mutagen and test sample, and M is the number of revertants per plate in the presence of mutagen (positive control). The numbers of spontaneous revertants (negative control, plates without diagnostic mutagen and test samples) were subtracted from numerator and denominator. The antimutagenic effect was considered weak, medium and strong when the inhibitory effect was less than 25%, 25-40% and more than 40%, respectively.

2.6. Effect of the extracts on the leakage of 260 nm absorbing material

The effect of acetone and methanol extracts of *P. ferrugineum* flowers on nucleic acid leakage (OD at 260 nm) was estimated as described by Carson et al [8]. Briefly, overnight broth cultures of *Bacillus cereus*, *Yersinia enterocolitica*, *Escherichia coli* and *Staphylococcus aureus* were harvested by centrifugation at 5000g for 10 mins. The pellet was washed with 2 ml PBS (pH 7.4) and was again centrifuged to retain the pellet. The pellet was resuspended in 1 ml PBS and OD at 620 nm was adjusted to ~0.3 by PBS for uniformity in different sets of experiments.

To the 50 μl of the above cell suspension, MIC and 0.5 MIC of the flower extracts of *Peltophorum ferrugineum* was added, volume was made upto 1 ml using PBS and incubated at 37 ℃. At different time intervals (0 min, 30 min, 60 min and 120 min), 50 μl mixture was added to 1.95 ml of PBS and OD was measured at 260nm against PBS blank. For control, only cell suspension (50 μl) was added to 1.95 ml PBS and read as above. The leakage of nuclear material to incubating medium was calculated in terms of % increase in OD at 260 nm in treatment over control at each incubation period.

2.7. Effect of the extracts on the bacterial respiration

The effect of acetone and methanol extracts of *P. ferrugineum* flowers on bacterial respiration was measured by estimating the total oxygen dissolved in the reaction mixture [9]. One hundred μl of cell suspension (as described in section 2.6) along with MIC and 0.5 MIC of extracts was made upto 2 ml by adding PBS, transferred to 125 ml reagent bottle, stirred for 5 min and the bottles were completely filled with deionized water. To this 1 ml each of manganese sulfate (48% w/v) and alkaline potassium iodide (sodium hydroxide, 50% w/v; potassium iodide, 15 % w/v) solutions were added. The precipitate formed was dissolved by addition of 1 ml of concentrated sulfuric acid, and free iodine liberated was estimated by titration against 0.005M-thiosulfate solution using starch as indicator. Each ml of 0.005 M-thiosulfate titrate was considered equivalent to 0.08 ppm of oxygen.

2.8. Effect of the extracts on bacterial Cell Wall

Scanning Electron Microscopy (SEM) was used to investigate

the effect of acetone and methanol extracts of *P. ferrugineum* flowers on bacterial cell wall [10]. Overnight cultures were centrifuged at 6000 rpm for 10 min at 4 ℃, washed twice with 0.1 M phosphate buffer (pH 6.5) and volume was made upto 0.5 ml with same buffer. The MIC of the extracts were added and the final volume was made upto 1 ml using phosphate buffer. The above cell suspension was incubated for 1 h and cells were harvested at 6000 rpm for 10 min at 4 ℃. The pellet was incubated in 1 % glutaraldehyde overnight at 0 ℃ and the cells were harvested at 6000 rpm for 10 min at 4 ℃. The cells were dehydrated in ethanol gradient (10–100 %) and coated with thin layer of gold using polaron SEM coating system. The cells were observed with a LEO 435 VP Scanning Electron Microscope at 20 KV attached to Mitsubishi Video copy processor. Photographs were taken using 35 mm Richo camera that was connected to monitor optically through fibre optics.

2.9. Statistical analysis

Since the MIC values were same in 4 experiments, the values were represented as such. The values for all other experiments were reported as mean±SD (n=3).

3. Results

The yield of hexane, ethyl acetate, acetone and methanol extracts from the flowers of *P. ferrugineum* were found to be 4.75, 6.31, 6.43 and 23.83 % (w/w), and the total phenolic content were 2.08, 1.7, 3.37 and 7.33 % as gallic acid equivalent (w/w), respectively. Acetone and methanol extracts inhibited the complete growth of tested organisms at the concentration range of 0.1% to 0.2%. However, hexane and ethyl acetate extracts were less effective and inhibited the complete growth of all the bacteria at 0.3% to 1.25% (Table 1).

Table 1

Minimum inhibitory concentration (MIC) of *Peltophorum ferrugineum* flower extracts

	MIC of flower extracts (%)			
	Hexane	Ethyl acetate	Acetone	Methanol
S. aureus	1.20	0.80	0.20	0.15
B. cereus	0.90	0.65	0.10	0.15
E. coli	1.25	0.80	0.15	0.20
Y. enterocolitica	0.60	0.30	0.10	0.10

Values are result of four replications where no growth was observed

Since acetone and methanol extracts inhibited the complete growth of all the four microorganisms at a lower concentration as compared to hexane and ethyl acetate extracts, further studies were done only with these two extracts. Both acetone and methanol extracts showed strong inhibition of mutagenicity induced by methyl methane sulfonate (MMS) and sodium azide in *Salmonella* tester strains. However, the degree of antimutagenic activity (82.6–99.3% inhibition) of these extracts varied among different tester strains (Table 2) and it was concentration dependent.

In the present study, we observed that with the increase in the time of incubation and concentration of *P. ferrugineum* flower extracts, the nucleic acid leakage from bacterial cells increases (Fig. 1). Similarly, increasing concentration of *P. ferrugineum* flower extracts showed increase in dissolved oxygen, which indicated the decrease in number of viable cell or inhibition of respiration (Table 3). Untreated reaction mixture showed dissolved oxygen from 0.09 to 0.11 ppm, whereas after addition of 0.5 MIC level of extract, the dissolved oxygen level were 0.30–0.81, and MIC level of extracts increased it to 0.6–1.6 ppm.

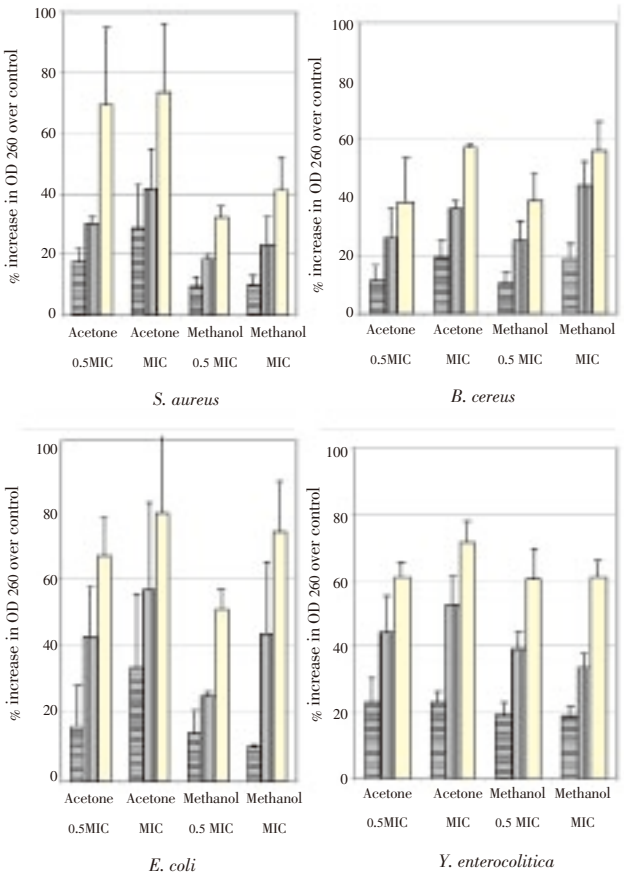


Figure 1. Effect of acetone and methanol extracts of *Peltophorum ferrugineum* on nucleic acid leakage (mean±SD, n=3) from bacteria (Horizontal lines in bar– 30 min, Vertical lines in bar– 60 min, No lines in bar– 120 min)

Disintegration of the cell wall was observed in scanning electron micrograph of B. cereus at MIC level treatment with both acetone and methanol extracts (Fig. 2). MIC of acetone extract was able to disintegrate cell wall in *S. aureus* and *E. coli*. But not many changes were observed in the cell wall of *Yersinia enterocolitica* after addition of the either of the extracts at MIC level. Untreated cells showed a continuous thin smooth cell wall, cell membrane and nuclear material and as the concentration of extracts increased cell wall lost

Table 2

Antimutagenic activity of *Peltophorum ferrugineum* flower extracts against *Salmonella* tester strains

	Concentration of flower extracts (mg/ plate)					
	Acetone			Methanol		
	1.25	2.5	5.0	1.25	2.5	5.0
Methyl methane sulfonate induced mutation						
TA 98	85.5±0.81	95.5±0.87	98.9±0.43	87.7±0.96	93.0±1.08	98.3±0.63
TA 100	87.3±0.28	94.5±0.65	97.6±1.04	84.6±0.89	94.5±0.64	98.0±0.67
TA 1531	86.6±0.75	95.5±1.00	98.1±0.81	82.6±0.78	88.6±1.10	96.5±0.64
Sodium azide induced mutation						
TA 98	89.6±0.96	93.5±0.79	97.5±0.91	85.2±1.29	92.7±0.85	95.5±0.81
TA 100	89.5±0.61	95.9±1.14	98.7±0.74	91.9±1.09	94.6±1.01	99.3±0.55
TA 1531	88.1±0.81	96.1±0.96	98.9±0.51	91.7±0.83	96.4±0.91	98.1±0.60

values are mean±SD of 3 replications

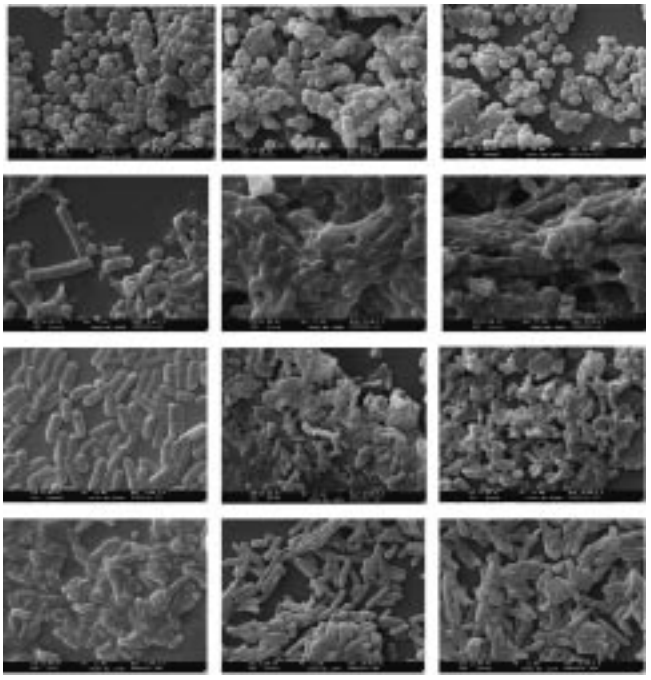
Table 3

Effect of *Peltophorum ferrugineum* flower extracts on bacterial respiration (in terms of dissolved oxygen in the reaction medium, ppm)

	Dissolved oxygen (ppm)				
	Untreated	Acetone		Methanol	
		0.5 MIC	MIC	0.5 MIC	MIC
S. aureus	0.09	0.30±0.02	0.68±0.08	0.71±0.07	1.38±0.01
B. cereus	0.11	0.52±0.07	1.03±0.15	0.81±0.05	1.6±0.39
E. coli	0.11	0.40±0.11	1.28±0.12	0.43±0.04	0.97±0.1
Y. enterocolitica	0.10	0.44±0.07	0.97±0.23	0.34±0.08	0.74±0.11

values are mean±SD of 3 replications

smoothness and uniformity.



Control Acetone extract treated Methanol extract treated

Figure 2. Effect of acetone and methanol extract of *Peltophorum ferrugineum* at minimum inhibitory concentration level on cell wall of bacteria (top to bottom– *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Yersinia enterocolitica*).

4. Discussion

The antibacterial compounds present in various flower extracts inhibited the growth of some of the foodborne pathogens tested in the present study, although the inhibition was variable depending on the extract and bacterium in question. Bhattacharjee et al [11] also observed that the antibacterial activity of various medicinal plants leaf extract varied depending on the type of extract as they reported organic extracts were more effective than aqueous extracts. Steenkamp et al [2] also reported variable antibacterial activity of water and methanol extracts of *P. africana*, as they inhibited complete growth of *S. aureus* at 0.36 and 0.2 % concentration, respectively. Gallic acid, ferulic acid and catechins have been reported to possess antimicrobial activities [1], and the extracts from the flower of *P. ferrugineum* have been found to contain substantial amount of phenolics and the major phenolic compounds are gallic acid, ferulic acid and catechin [12]. Kaisoon et al [13] also reported phenolics to be responsible for biological activities of various Thai edible flowers. The differential polyphenolic content of *P. ferrugineum* flower extracts may be responsible for their variable antibacterial effect in the present study.

The strong antimutagenic activity shown by acetone and methanol extracts against tester strains (>40% inhibition of mutagenicity induced by mutagens) varied with the tester strain and mutagen used, and was in agreement with the finding of other workers who have reported concentration dependent antimutagenic activity [14, 15].

The antibacterial activity of various antimicrobials such as phenols, flavonoids, terpenoids, coumarin and

alkaloids present in natural preservatives is due to several mechanisms, including cell wall disintegration and degradation of genetic material. In the present study, we observed that the nucleic acid leakage from bacterial cells increases with time of incubation and concentration of *P. ferrugineum* flower extracts (Fig. 1). Probably interaction of the compounds present in flower extracts such as gallic acid, ferulic acid and catechin [12] with the bacteria causes the nucleic acid (260 nm absorbing material) to leach out to the incubating medium. Extracts of *Eupatorium hecatanthum* and *Pterocaulon polystachium* also contain compounds, which interact with DNA [16]. The denaturation of DNA and loss of OD260 materials by phenolic rich extracts from the fruits of *Livistona chinensis* have been observed in *S. aureus* also [17].

Various phenolic compounds inhibited dehydrogenase activity and inhibited respiration by the bacteria [18]. Probably the *P. ferrugineum* extracts inhibit the respiration of bacteria, cause loss of OD260 materials and disintegrate the cell wall due to the presence of phenolic compounds [12].

In conclusion, this study showed that acetone and methanol extracts from the flowers of *P. ferrugineum* were effective antibacterials against the foodborne pathogens and also showed strong antimutagenic activity. The antibacterial activity of these phenolics rich extracts may be due to the disintegration of cell wall, leakage of nuclear material and inhibition of bacterial respiration. Thus, the flowers can find application as natural food preservative and as nutraceutical, but their application in food system and toxicological aspect needs further study.

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Conflict of interest statement

We declare that we have no conflict of interest.

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