

## Antimicrobial Activities of Rhizome Extracts of Mango Ginger (*Curcuma amada*) against Food Spoilage by Bacterial Isolates of Fruits, Vegetables and Oilseed

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### ABSTRACT

The present study aimed at isolation and identification of food spoilage bacterial isolates of fruits, vegetables and oilseeds. These isolates were screened for gram reaction, catalase and other biochemical characteristics. Results revealed that these isolates belong to the genus *Proteus*, *Staphylococcus*, *Enterobacter*, *Serratia*, *Streptococcus* and *Alcaligenes*. These isolates were further studied for sugar fermentation profiles and results confirmed that the majority of them were able to metabolize glucose, sucrose, fructose and mannitol, while only one isolate was able to metabolize lactose. Finally, different mango ginger extracts were screened for their antimicrobial activity against spoilage bacterial isolates by agar well and disc diffusion assay. Ethanolic extracts were found to be effective against *Staphylococcus* and *Serratia* spp. followed by acetone and hexane extracts. All other isolates were found to be resistant to all three extracts.

*Keywords* : *Curcuma amada*, Agar well diffusion method, Disc diffusion method, Biochemical characterization

SPICES are used in food preparations to add flavor and color. They also possess medicinal properties. They are used to treat ailments and prevent diseases (Lai and Roy, 2004). Spices are employed in food preservation because of their antimicrobial properties, e.g., pickles, bread and rice (Shelef, 1984 and Davidson *et al.*, 2013). Spices such as cloves, cinnamon, garlic, bay leaves, black cumin, mustard, rosemary, thyme, allspice etc., are used in therapeutic formulations as they possess antimicrobial activities (Goreza, 2003). *Curcuma amada* Roxb. popularly known as mango ginger because of its morphological similarity to *Zingiber officinale* and *Curcuma longa* and raw mango flavor. It is a perennial herb commonly found in hilly areas of South India and North-Eastern region of India. *C. amada* is a rich source of starch, fiber and consists of car-3-ene and cis-ocimene responsible for raw mango flavor (Rajkumari and Sanatombi, 2017).

Mango ginger is a rich source of fiber, starch and some phytochemicals having biological and pharmacological properties. These biological properties include antimicrobials, antioxidants, anti-inflammatory, cytotoxicity, anti-allergic activity, biopesticide

activity, etc., due to the presence of chemical compounds such as phenolic acids, volatile oils, curcuminoids and terpenoids like difurocumenonol, amadannulen and amadaldehyde (Policegoudra *et al.*, 2011). Extracts from medicinally important plants such as turmeric were studied for its antimicrobial activity and also showed increased shelf life of tomato in biopreservation (Sadananda *et al.*, 2011). A novel antimicrobial compound difurocumenonol was identified and isolated from mango ginger extracts having high antimicrobial activity against both Gram-positive and Gram-negative bacteria (Policegoudra *et al.*, 2006). Studies of different extracts of hexane, chloroform, acetone and methanol have been found highly antibacterial against *Bacillus cereus*, *B. subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Enterobacter fecalis* and *Salmonella typhi*. Phenolics of mango ginger rhizome have been reported to exhibit antimicrobial activities. Volatile oils such as myrcene and pinene from mango ginger rhizome showed antifungal properties and were studied against *Aspergillus niger*, *A. terreus*, *Fusarium monoliforme* and *F. falcatum* (Policegoudra *et al.*, 2007a). Vaibhavi *et al.* (2019) had studied

physico chemical parameters and shelf life of value added products from mango ginger and concluded absence of coliforms and molds. Thus, value added products with increased shelf life can be developed from mango ginger.

The objective of the study was to isolate and identify the food spoilage bacteria from various sources by assessing their morphological and biochemical characteristics. Further, mango ginger extracts were screened for their antimicrobial activity against spoilage bacterial isolates by agar well and disc diffusion assay.

## MATERIAL AND METHODS

### Sample Collection

Spoiled fruits and vegetables were collected from local markets in Bengaluru and were washed. Isolation was attempted from spoiled papaya, apple, pomegranate, tomato, beans, brinjal and groundnuts.

### Isolation and Purification of Spoilage Bacteria

A known quantity (one gram) of a spoiled portion of fruits and vegetables was transferred to 10 mL sterile saline (0.85 %) and one mL aliquot from this suspension was serially transferred to the following saline blanks to get  $10^{-5}$  dilution. Aliquots (one mL) of each dilution was pipetted out to sterile Petri plates and nutrient agar medium (NA) was poured. The plates were incubated at 28 °C for 48h. The colonies with different morphology were randomly selected and streaked on nutrient agar plates to get pure bacterial colonies. Isolates were obtained after successive transfers and were preserved in 20 per cent glycerol stocks.

### Preliminary Identification of Spoilage Bacteria

The pure bacterial isolates were tested for Gram reaction, endospore formation and catalase activity.

### Gram Reaction

Gram staining was the first step in the preliminary identification of bacterial isolates. It was performed by adding crystal violet (primary stain) to the heat-fixed smear of bacterial culture for one min, washed and followed by adding iodine. Iodine was retained

for one min and washed. The slide was dipped in ethanol (95 %) for 45 sec to one min. Then safranin was added over smear as a counter stain for one min and washed. The slides were observed under oil immersion (Bartholomew and Mittwer, 1952).

### Endospore Formation

As old cultures produce endospores, 30 days old bacterial cultures were subjected to test for endospore formation. The smears of isolates were prepared on clean slides, air-dried and heat-fixed. Blotting paper was placed on the smear and flooded with malachite green (5% aqueous). These slides were steamed for five to seven minutes by adding more stains to the smear from time to time to avoid drying. Then, the slides were washed, counterstained with safranin for 30 seconds and observed under oil immersion. (Murray *et al.*, 1994).

### Catalase Activity

A loop full of each isolate was placed on cleaned glass slides and three to four drops of hydrogen peroxide (3 %) were added. Both were mixed with the help of inoculation needle and observed for the appearance of effervescence. (Dhameliya *et al.*, 2020).

### Biochemical Characterization

#### Acid and Gas Production

The sterilized glucose broth with pH indicator (phenol red) and an inverted Durham's tube test tubes were inoculated with bacterial isolates to observe for gas production. These tubes were incubated for 48 hours at 32 °C to observe a color change (due to acid production) and the appearance of bubbles in Durham's tube (due to gas production) (Seeley and Vademark, 1970).

#### Methyl Red (MR) & Voges Proskauer (VP) Test

The bacterial isolates were inoculated in MRVP broth (pH 6.9) and incubated at 32 °C for 48 hours. After incubation, five drops of methyl red indicator was added for the MR test, and observed for color change. Naphthol solution (12 drops) and 2-3 drops of potassium hydroxide (40 %) were added to broth and

a color change was observed for the VP test (Clarke and Kirner, 1941).

### **Gelatin Hydrolysis**

The bacterial isolates were stab inoculated in nutrient gelatin tubes and incubated at 32 °C for four days. After incubation, tubes were placed in refrigerator at 4 °C for 15 minutes to examine the liquefaction of gelatin which was considered as a positive result, tubes that remained in solid-state were scored as negative (Ewing, 1962).

### **Indole Production**

It was performed by inoculating bacterial isolates in tryptone broth and incubating at 32 °C for 48 hours, followed by the addition of Kovac's reagent. The development of cherry red color in the top layer of the tube was a positive result (Skinner and Lovelock, 1980).

### **Citrate Utilization**

The bacterial isolates were inoculated in Simmon's citrate agar slants and incubated at 32 °C for 48 hours. The slant cultures were observed for growth and coloration of the medium. Growth on the surface of medium and appearance of blue color is considered as positive and no growth and no change in color (green) was scored as negative result. (Skinner and Lovelock, 1980)

### **Hydrogen Sulfide Production**

The bacterial cultures were inoculated in SIM (sulfide indole motility) agar stabs inoculation and incubated at 32 °C for 48 hours. After incubation, tubes were observed for the presence or absence of black coloration along the line of inoculation (Skinner and Lovelock, 1980).

### **Carbohydrate Fermentative Profiles of Bacterial Isolates**

The bacterial isolates were inoculated in nutrient broth with an addition of specific carbohydrates (glucose, sucrose, fructose, lactose and mannitol) and phenol red as pH indicator, followed by incubation at 32 °C

for 48 hours. The change in color of broth from red to yellow (due to the production of acid) was a positive result (Kavitha *et al.*, 2016).

### **Mango Ginger Rhizome Extract Preparation**

Rhizomes of mango ginger were purchased from the local market in Bengaluru, India. These fresh rhizomes were washed with 2 per cent sodium hypochlorite solution followed by sterile distilled water, sliced and dried. Ten grams of dried rhizomes were homogenized in a blender and extracted with ethanol (100 mL), acetone (100 mL), hexane (100 mL) and sterile distilled water (100mL), then kept at 110 rpm in a rotatory shaker for 72 hours at room temperature. The extracts were separated by filter paper (Whatman no. 1) and evaporated by a rotary evaporator to get 10 mL volume. The extracts were kept in sterile vials at 4 °C until use. (Dezoysa *et al.*, 2019)

### **Inoculum Preparation**

Bacterial isolates were inoculated in 100 mL nutrient broth and incubated in a rotatory shaker at 100 rpm for 24-48 hours at 30 °C to get the density of  $10^5$  cells per mL.

### **Antimicrobial Activity of Mango Ginger Extracts against Isolated Bacteria**

Agar well diffusion method (Nathan *et al.*, 1978) and disc diffusion method (Bauer *et al.*, 1966) were used for the detection of antimicrobial activity of different extracts of mango ginger rhizome. Five per cent acetic acid was taken as a reference standard for the antibacterial test. Nutrient agar medium-containing plates were prepared. Bacterial broth cultures were spread in separate plates in triplicates. For the agar well method, wells (7 mm diameter) were made using sterile cork borer and different extracts (50 µL) (ethanol, acetone, hexane and aqueous) were introduced into the wells. Ethanol, acetone, hexane and sterile distilled water were kept as negative control and 5 per cent acetic acid as a positive control. The plates were allowed to stand for an hour to allow diffusion of extracts from wells and incubated for 48 hours at 30 °C. Results were recorded after every 24 hours.

For the disc diffusion method, discs (7 mm diameter) were made using Whatman filter paper and sterilized. 60 µL of different extracts (ethanol, acetone, hexane and aqueous) was added to each disc and placed on bacterial isolates seeded nutrient agar medium plates. Ethanol, acetone, hexane and sterile distilled water were kept as negative control and 5 per cent acetic acid as positive control. These plates were allowed to stand for one hour to allow diffusion of extracts from discs and incubated for 48 hours at 30 °C. Results were recorded after every 24 hours.

## RESULTS AND DISCUSSION

### Isolation and Purification of Spoilage Bacteria

The spoilage bacteria were isolated by following routine microbiological procedures on nutrient agar medium, suitable for the growth of all bacteria. A total of seven isolates were observed with distinguishable colony characteristics, size varying from small to large and color ranging from colorless to red-pigmented colonies. These colonies were purified and preserved after repetitive sub-culturing.

### Preliminary Identification of Spoilage Bacteria

The purified isolates were screened for morphological characterization, where three isolates were Gram-positive, four of them were Gram-negative and all of them were catalase-negative. The isolates with varied cell arrangements from independent coccus / rods to

cells of two / four / group or short / longchains are summarized (Table 1). These isolates were further analyzed for biochemical characteristics.

### Biochemical Characterization

Three of the isolates fermented glucose with gas production. None of the isolates had hydrolyzed starch and gelatin. Isolates were negative for the methyl red test, Voges-Proskauer's test and hydrogen sulfide production, but three isolates were positive for the citrate test showing growth and color change (Table 2).

### Sugar Fermentation Profile

These isolates were further studied for sugar fermentation capabilities and were able to ferment glucose, sucrose, fructose, lactose and mannitol (Table 3). All the isolates were capable of fermenting fructose with no gas production. Three isolates could use glucose and sucrose, with no gas production. Four isolates fermented mannitol with gas production and only one isolate was able to ferment lactose.

### Antimicrobial Activity of Mango Ginger Extracts against Bacterial Isolates

Antimicrobial activity of different extracts (ethanolic, acetone, hexane and aqueous) of mango ginger rhizome was tested against spoilage bacteria using the agar well method and disc diffusion method. Except for aqueous extracts, all extracts were effective

TABLE I  
Morphological characteristics of bacterial isolates (+ Positive, - Negative)

Isolates	Colony characteristics			Morphological characteristics		
	Size	Shape	Colour	Cell shape	Cells arrangement	Endospore formation
AP.B1	Large	Round	Transparent	Bacilli	Independent	-
PO.B1	Large	Round	Dull white	Cocci	In clusters	-
PA.B1	Large	Round	Colorless	Bacilli	Independent	-
PA.B2	Small	Round	Red	Bacilli	Independent	-
TO.B1	Small	Round	Dull White	Cocci	In chains	-
TO.B2	Small	Round	White	Bacilli	Independent	-
TO.B3	Medium	Round	White	Cocci	In chains	-

TABLE 2  
Biochemical characterization of bacterial isolates (+ Positive, - Negative)

Isolates	Gram reaction	Catalase	MR	VP	Citrate utilization	Hydrolysis of Starch	Hydrolysis of Gelatin	Production of Indole	Production of H <sub>2</sub> S
AP.B1	-	-	-	-	+	-	-	-	-
PO.B1	+	-	-	-	-	-	-	-	-
PA.B1	-	-	-	-	+	-	-	+	-
PA.B2	-	-	-	-	+	-	-	+	-
TO.B1	+	-	-	-	-	-	-	-	-
TO.B2	-	-	-	-	-	-	-	-	-
TO.B3	+	-	-	-	-	-	-	-	-

TABLE 3  
Sugar fermentation profiles of bacterial isolates  
(+ Positive, - Negative)

Isolates	Glucose	Sucrose	Lactose	Fructose	Mannitol
AP.B1	+	+	-	+	+
PO.B1	-	-	-	+	+
PA.B1	+	+	+	+	+
PA.B2	+	+	-	+	+
TO.B1	-	-	-	+	-
TO.B2	-	-	-	+	-
TO.B3	-	-	-	+	-

against POB1, where ethanolic extract has shown a maximum zone of inhibition followed by acetone and hexane extracts. Ethanolic extracts were also found to have an inhibitory effect against PAB2. These results were compared with the negative control (ethanol, acetone, hexane and sterile distilled water), which had shown no zone of inhibition. Five per cent acetic acid was used as positive control showing maximum zone of inhibition against all the isolates in agar well and disc diffusion method.

Foods such as fruits, vegetables, oilseeds, etc., are constantly exposed to spoilage and pathogenic

TABLE 4  
Antimicrobial activity of *Curcuma amada* (mango ginger) rhizome extracts  
against bacterial isolates by Agar well method

Extracts	Inhibition zone (in mm) against bacterial isolates (includes the diameter of well 7 mm)						
	APB1	POB1	PAB1	PAB2	TOB1	TOB2	TOB3
Acetone extract	0.00 (0.70)	12.41 (3.58)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Acetone	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Hexane extract	0.00 (0.70)	11.83 (3.50)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Hexane	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Ethanol extract	0.00 (0.70)	14.91 (3.91)	0.00 (0.70)	10.66 (3.34)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Ethanol	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	8.66 (3.02)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Aqueous extract	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Sterile distilled water	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
5% acetic acid	46.00 (6.81)	49.66 (7.08)	38.33 (6.22)	54.00 (7.38)	39.33 (6.33)	46.83 (6.88)	49.75 (7.08)
CD (95%)	1.56	1.74	1.41	1.75	1.44	1.58	1.63
CV	0.29	5.17	2.07	0.88	4.01	1.02	0.24
SEM±	0.67	0.75	0.61	0.76	0.62	0.68	0.71

TABLE 5  
Antimicrobial activity of *Curcuma amada* (mango ginger) rhizome extracts against bacterial isolates by Disc diffusion method

Extracts	Inhibition zone (in mm) against bacterial isolates (includes the diameter of disc 6 mm)						
	APB1	POB1	PAB1	PAB2	TOB1	TOB2	TOB3
Acetone extract	0.00 (0.70)	8.10 (2.93)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Acetone	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Hexane extract	0.00 (0.70)	10.25 (3.27)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Hexane	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Ethanol extract	0.00 (0.70)	7.00 (2.73)	0.00 (0.70)	8.00 (2.91)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Ethanol	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Aqueous extract	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Sterile distilled water	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
5% acetic acid	7.66 (2.85)	13.33 (3.72)	11.25 (3.41)	13.00 (3.66)	12.33 (3.58)	15.66 (4.02)	12.33 (3.60)
CD (95%)	0.55	0.87	0.69	0.88	0.73	0.85	0.73
CV	1.30	21.93	4.70	1.15	0.44	1.99	2.83
SEM ±	0.24	0.38	0.30	0.38	0.32	0.37	0.32

microorganisms. The present study deals with the isolation of such spoilage and pathogenic bacteria from different food samples. A total of seven bacterial isolates were obtained and identified as *Proteus* spp. (APB1), *Staphylococcus* spp. (POB1), *Enterobacter* spp. (PAB1), *Serratia* spp. (PAB2), *Streptococcus* spp. (TOB1 & TOB3) and *Alcaligenes* spp. (TOB2) based on Gram reaction and other biochemical studies. These isolates were further studied for antimicrobial activity of mango ginger rhizome extracts against it by agar well assay and disc diffusion assay. The results showed that ethanolic extract of *Curcuma amada* rhizome exhibited significant antibacterial activity against Gram-positive and Gram-negative bacteria, whereas the aqueous extract has the weakest activity against these microorganisms. Mango ginger rhizome possesses antibacterial activity against food spoilage microorganisms. It can be due to the presence of different bioactive compounds such as free and bound phenolics, terpenoids, curcuminoids and a novel antibacterial compound, difurocumenonol (Policegoudra *et al.*, 2011).

Mango ginger rhizome has been studied for the presence of phytochemicals and essential oil responsible for its biological activities. The antimicrobial activity of mango ginger rhizome was investigated against Gram-positive and Gram-negative bacteria which can be due to the presence of phenolics, terpenes and curcuminoids. The present study concludes that the mango ginger rhizome extracts were effective against Gram-positive and Gram-negative bacteria which shows the potential of bioactive compounds present. Also, further investigations are required to study the phytochemicals present in mango ginger rhizome to utilize it in food preservation.

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