International Journal of Current Science Research
www.drbgrpublications.in

Screening of Rutin and Isolation, Identification of Foodborne Drug Resistance Biofilm Producing *Staphylococcus aureus* and *Escherichia coli* from Fast Food

[1]Karuppasamy G, [1]Sakthivel A, [1]Kiruthika D and [2*]Muthulakshmi K

[1]*PG & Research Department of Microbiology, V.H.N.Senthikumara Nadar College (Autonomous), Virudhunagar, Tamilnadu*

[2]*Assistant Professor, PG & Research Department of Microbiology, V.H.N.Senthikumara Nadar College (Autonomous), Virudhunagar, Tamilnadu*

[*]*Corresponding Author e-mail id: muthulakshmi.k@vhnsnc.edu.in*

Abstract

This study focused on the health concerns that arise foodborne drug resistance Biofilm producing Staphylococcus aureus and Escherichia coli isolated from several fast food. Public health and the food business worldwide are grappling with the establishment and spread of multi-drug resistance among these dangerous diseases. Moreover, the development of biofilms by foodborne pathogens has gathered significant interest in recent times because of the possible hazards associated with it, such as heightened susceptibility to antibiotics and the generation of virulence factors that result in food illness. This research explores the antibacterial activity of rutin, a flavonoid compound, isolated from banana leaves (Musa spp.) and its ability to suppress biofilm development by S.aureus and E.coli in fast foods. Bacteria were isolated from chicken fried rice and chicken burger samples homogenized and centrifuged at 4500 rpm for 15 minutes with culture techniques and characterized using biochemical tests, in which S. aureus and E. coli were the major isolates. Rutin was isolated from banana leaves with ethanol, and its phytochemical content was verified by ferric chloride test for the presence of phenolic compounds. The rutin extract was then analyzed using HPLC.

Keywords: Rutin, HPLC, Fast food, Drug resistance bacteria, Public health

Introduction

Rutin, also referred to as rutoside, quercetin-3-rutinoside, or sophorin, is a naturally occurring flavonoid found in various plant species (Kreft *et al*., 1999). Rutin exhibits limited water solubility, but its solubility increases in alcoholic solutions. Notably, rutin possesses

several valuable pharmacological properties that confer health benefits (Habtemariam and George, 2015).

Rutin exhibits multifaceted therapeutic potential, offering promise as an antioxidant, antimicrobial, antifungal, antiallergic, and anticancer agent. Additionally, it may be beneficial in managing diabetes and hypertension (Shama and Patel, 2013; Frezza *et al*., 2018). Buckwheat is a rich source of rutin, with the flavonoid being present in various parts of the plant, including the groats, seeds, leaves, and flowers (Hussain *et al*., 2017).

The smoke tree (Rhus cotinus), an inedible shrub, boasts the highest recorded rutin content, with a remarkable 10.5% concentration found in its dried fruit (Atanassova and Bagdassarian, 2009). Bananas are among the most extensively cultivated, distributed, and consumed fruits globally, particularly in tropical and subtropical regions (Laillyza *et al*., 2014). Bananas are a nutrient-rich food crop, boasting an impressive profile of essential minerals, vitamins, carbohydrates, flavonoids, and phenolic compounds, making them a valuable dietary staple worldwide (Imam and Akter, 2011). Rutin, a potent antioxidant with numerous health benefits, is hindered by its high production costs. To make rutin more accessible, it's essential to identify an abundant, affordable source. Research has revealed that Musa paradisiaca leaves, closely related to M. balbisiana, contain rutin in their crude extracts and fractions (Coskun, 2016).

Globally, public health agencies face mounting concerns regarding food safety assurance, driven by the increasing globalization of food markets and the rising trend of consuming meals prepared outside the home. The global production and consumption of processed meat products have risen steadily in recent years, driven by their high nutritional value and convenience. However, these products can pose a significant public health risk due to the potential presence of foodborne pathogens, which can cause illness, intoxication, and even death.

Staphylococcus aureus is a leading cause of food poisoning worldwide, contaminating various foods, including ready-to-eat vegetables and processed meats, and producing harmful enterotoxins. Meanwhile, *Escherichia coli*, particularly the psychrotrophic strain *E. coli* O157:H7, poses a significant public health risk due to its ability to grow on minimally processed vegetables and processed meats at refrigerated temperatures (4-12°C), leading to

severe foodborne illnesses like hemorrhagic colitis. Traditional methods for detecting foodborne pathogens like *S. aureus* and *E. coli* in food involve using selective media like Baird-Parker and MacConkey agar, followed by biochemical tests to identify suspicious colonies. However, these conventional methods are time-consuming, labor-intensive, and often yield ambiguous results, particularly with field isolates. To ensure microbiological safety throughout the food production chain, rapid and sensitive detection methods are crucial. In recent years, numerous molecular-based detection methods have been developed, offering promising alternatives to traditional approaches.

The primary goal of this study is to detect, isolate, and enumerate *Staphylococcus aureus* and *Escherichia coli* in ready-to-eat vegetable salads and meat luncheon products. The identification of these pathogens will be achieved through biochemical reactions and molecular analysis using PCR-based 16S rRNA gene sequencing. The rise and spread of multi-drug resistance among foodborne pathogens pose a significant threat to both the food industry and public health worldwide. Research indicates that the use of antibiotics in animal agriculture has accelerated the development of resistant bacterial strains, which can be transmitted to humans through the food chain (Chang *et al*., 2015).

Ready-to-eat fast foods are vulnerable to contamination by pathogens due to inadequate handling and sanitation practices. Building on previous research highlighting the potential of phytochemicals as biofilm control agents (Ahmad *et al*., 2014), this study investigates the efficacy of the flavonoid Rutin as a broad-spectrum biofilm inhibitor against mono- and multi-species biofilms formed by drug-resistant *E. coli* and Methicillin-resistant *Staphylococcus aureus* (MRSA).

Materials and Methods

Sample collection: The two types of fast foods were collected from local shop in Virudhunagar, Tamil Nadu. The sample banana leaves was purchased from the local shop in Virudhunagar, Tamil Nadu. The banana leaves was carefully washed and air dried at room temperature for 5 days and pulverized to a fine powder with the use of a sterilized mixer grinder.

Bacterial isolation: The fast food samples were homogenized separately (20g of sample and 80ml of normal saline). Homogenized samples were put into 15ml centrifuge tube, samples are

centrifuged at 4500rpm for 10minutes. The supernatant was collected after centrifugation and plated on EMB agar, Mannitol salt agar. The agar plates are incubated at respective temperature for 24-48 hours.

Identification

Gram staining: The first step in characterizing the bacteria was to perform Gram staining. This technique involves applying a series of dyes to a bacterial smear on a glass slide. The bacteria were first stained with crystal violet, followed by treatment with Gram's iodine. The slide was then decolorized with ethanol and counterstained with safranin. The resulting stain determined whether the bacteria were Gram-positive or Gram-negative.

Biochemical characterization

Indole Test: The indole test was conducted to determine if the bacteria could degrade the amino acid tryptophan. A tryptophan-rich medium was inoculated with the bacteria, which were then incubated at 37°C for 24 hours. Kovac's reagent was added to the culture, and the appearance of a red-colored product indicated a positive reaction.

Methyl Red Test: The methyl red test was used to detect acid production by the bacteria. The bacteria were grown in a glucose-rich medium, which was then inoculated into a methyl red broth. The broth was incubated at 37°C for 24 hours, after which methyl red solution was added. A color change indicated a drop in pH, signifying a positive result.

Voges proskauer Test: The Voges Proskauer test was conducted to detect the production of acetoin by the bacteria. The bacteria were grown in a glucose-rich medium, which was then inoculated into a Voges-Proskauer broth. The broth was incubated at 37°C for 24 hours, after which Barritt's reagent was added. A color change indicated a positive reaction.

Citrate Utilization Test: The citrate utilization test was used to determine if the bacteria could utilize citrate as a sole carbon source. The bacteria were streaked onto Simmon’s citrate agar slants, which were then incubated at 37°C for 24 hours. A color change indicated a positive reaction.

Triple Sugar Iron Test: The triple sugar iron test was conducted to assess the ability of the bacteria to ferment glucose, lactose, and sucrose. The bacteria were inoculated into a triple sugar iron agar slant, which was then incubated at 37°C for 24-48 hours. A color change

indicated sugar fermentation, while the production of hydrogen sulfide gas caused blackening of the medium.

Catalase Activity Test: The catalase activity test was used to detect the presence of catalase enzyme in the bacteria. A few drops of hydrogen peroxide were added to the bacterial culture, and the release of oxygen gas was indicated by the formation of white bubbles.

Coagulase Test: The coagulase test is a diagnostic procedure used to identify *Staphylococcus aureus*, which produces the enzyme coagulase. The test involves mixing a bacterial colony with rabbit plasma containing EDTA, which is then incubated at 37°C for 1-4 hours. A positive result is indicated by the formation of a clot or gel-like substance, which occurs when the coagulase enzyme produced by *S. aureus* converts the fibrinogen in the plasma into fibrin, causing it to coagulate. In contrast, *Staphylococcus epidermidis* and other coagulase-negative *staphylococci* do not produce coagulase and therefore do not form a clot, resulting in a negative test result.

Antibiotic Susceptibility Test: The antibiotics susceptibility test, also known as the Kirby-Bauer test, is a diagnostic procedure used to determine the effectiveness of various antibiotics against a specific bacterial isolate. The test involves inoculating a bacterial culture onto a Mueller-Hinton agar plate, which is then incubated at 37°C for 24 hours. Antibiotic discs containing specific concentrations of antibiotics are placed on the agar surface, and the plate is incubated for an additional 24 hours. The zone of inhibition, which is the clear area around each disc where bacterial growth is inhibited, is measured in millimeters. The size of the zone of inhibition is compared to a standard chart to determine the susceptibility of the bacteria to each antibiotic, with larger zones indicating greater susceptibility.

Preparation of ethanolic extract of banana leaves: A quantity of 20g of air dried powder is immersed in 100ml of ethanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 165-170 rpm for 4 days. Then the extract was filter by using whatman no1 filter paper.

Confirmation test for rutin: Add a few drops of 1% ferric chloride solution to the extract. A green or blue color indicates the presence of phenolic compounds, including rutin.

Results

Sample collection

Fig 1: Fast food

Fig 2: Banana leaves

Bacterial isolation

Table: Isolation of bacteria from fast food sample

Strain Name	Colony Morphology in selective media
GY	Golden yellow color colonies in Mannitol Salt Agar
MG	Metallic Green shine colonies in EMB agar

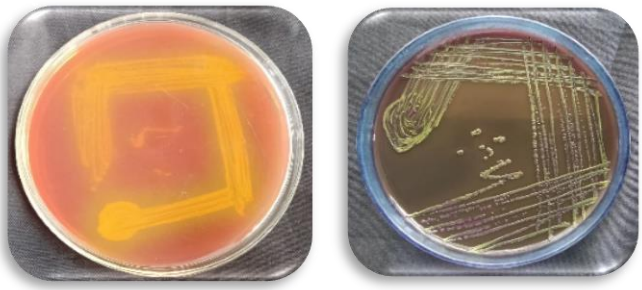

Fig 3: Isolation of bacteria from fast foods

Identification

Gram staining: Two strains were confirmed by gram straining, they are

GY - Gram positive grape like cluster

MG - Gram negative rod shaped

Biochemical characterization

Tests & Strains	GY	MG
Indole	-ve	+ve
Methyl red	+ve	+ve
VP	+ve	-ve
Citrate	+ve	-ve
TSI	A/A No gas formed	A/A Gas formed
Oxidase	-ve	-ve
Catalase	+ve	+ve

Coagulase test: The *Staphylococcus aureus* species was confirmed by positive reaction of coagulase test.

Fig 4: Coagulase test (Slide method)

Preparation of ethanolic extraction of *Musa acuminata* leaves

Fig 5: Ethanolic extract of banana leaves

Confirmation test for rutin

The rutin compound in the extract was confirmed by the method of phytochemical ferric chloride test.

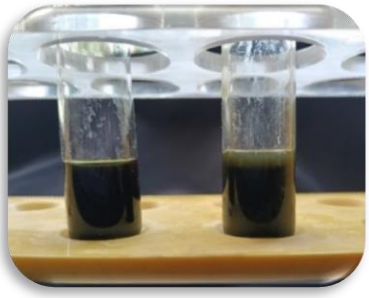

Fig 6: Flavonoid test

Future aspects

Further, rutin compound will be identified and imperturbable by using TLC and HPLC method. The highly biofilm producing and resistance bacteria will be further identified by using 16S rRNA gene sequencing.

Conclusion

In summary, the findings indicate that foods that are ready to eat have the potential to become contaminated by microorganisms and can harbor infections that are resistant to drugs and build biofilms. The study showed that rutin can lower biofilms. It is anticipated that the use of rutin in all conducted trials will not lead to pathogens developing resistance, given the reduction of biofilm was not caused by growth inhibition. The presence of saccharide groups in rutin may cause the sensitizer (MB) to be oriented toward the polysaccharide domains of the bacterial cell walls, which will aid in the compound's penetration of the cells.

Reference

A. Rajić, L.A. Waddell, J.M. Sargeant, S. Read, J. Farber, M.J. Firth, A. Chambers *J. Food Prot*., 70 (5) (2007), pp. 1286-1294.

Ahmad, I., Husain, F. M., Maheshwari, M., and Zahin, M. (2014). Phytochemicals as biofilm inhibitors: A promising strategy to combat bacterial biofilms. *Journal of Pharmacy and Pharmacology*, 66(11), 1471-1485.

Atanassova, M., and Bagdassarian, V. (2009). Rutin content in plant raw materials. *Pharmaceutical Chemistry Journal*, 43(7), 361-363.

Chang, Q., Wang, W., Regev-Yochay, G., Lipsitch, M., and Hanage, W. P. (2015). Antibiotics in agriculture and the risk to human health: How worried should we be? Evolution, Medicine, and Public Health, 2015(1), 219-227.

Coskun, O. (2016). Phytochemical analysis and antioxidant activity of Musa paradisiaca leaves. *Journal of Pharmacy and Pharmacology*, 68(8), 1050-1058.

Frezza, C., et al. (2018). Rutin: A flavonoid with multifaceted therapeutic applications. *Journal of Pharmacy and Pharmacology*, 70(8), 1050-1063.

G. Amagliani, C. Glammarini, E. Omicciolo, G. Brandi, M. Magnami, Food Control, 18 (2007), pp. 1137-1142.

Hussain, S., *et al.* (2017). Buckwheat: A nutrient-rich pseudocereal for food security and sustainable agriculture. *Journal of Food Science and Technology*, 54(4), 1088-1097.

Imam, M. Z., & Akter, S. (2011). Banana: Nutritional and pharmacological significance. *Journal of Food Science and Technology*, 48(3), 268-275.

J. Kennedy, P. Wall Food safety challenges M. Storss, M. Devoluy, P. Cruveiller (Eds.), Food safety Handbook: Microbiological Challenges, Bio-Merieux education, France (2007), pp. 8-19.

J.M. Janda, S.L. Abbott J. Clin. Microbiol., 45 (2007), pp. 2761-2764.

Kreft, S., et al. (1999). Rutin. In: I. P. Johnston (Ed.), Encyclopedia of Food Science and Nutrition (pp. 509-512). Academic Press. Habtemariam, S., & George, M. (2015).

L. Sommer, Y. Kashi, *J. Food Prot*., 66 (2003).

Laillyza, W. M., et al. (2014). Physicochemical properties of banana fruit at different ripening stages. *Journal of Food Science and Technology*, 51(10), 2934-2943.

S. Sanz, M. Gimenez, C. Olaret *J. Food. Prot.*, 66 (2003), pp. 2203-2209.

Shama, S. K., & Patel, M. S. (2013). Rutin: A review of its pharmacological and clinical applications. *Journal of Pharmacy and Pharmacology*, 65(8), 1057-1067.

The chemical and pharmacological basis of the medicinal plants used in Ethiopian traditional medicine. *Phytotherapy Research*, 29(9), 1275-1285.