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Antimicrobial effect of eatables against specific Gut Microbes

Jyotsana Singh*, Farha Shaikh, Rajvinder Kaur, Lubna Anis, Anshu
Department of Microbiology, Krishna College of Science and Information Technology, Bijnor NAAC Accredited Affiliated to M.J.P. Rohilkhand University Bareilly, Uttar Pradesh, India

Abstract
A range of chronic diseases, including cancer, inflammatory bowel disease, obesity, type 2 diabetes, and cardiovascular disease may be prevented in part by maintaining a healthy gut microbiota, according to current studies. Studies showing the significance of foods like ginger, garlic, white radish, onion, raw papaya, and green chili in supporting gut flora have also made it clear that diet significantly influences the microbiome. The highest concentrations of phytochemicals may be found in fruits and vegetables. The data showed that alkaloids, flavonoids, glycosides, carbohydrates, and phenolic compounds are present in ginger, garlic, onion, raw papaya, green chili, and white radish. *Bifidobacterium dentium*, *Lactobacillus buchneri*, *Lactobacillus formosensia*, *Lactobacillus agilis*, and *Aerococcus suis* were the major strains we employed in the study. Ginger and green chili have the same zone of inhibition which is 10.8 mm for *Bifidobacterium dentium*. A 9 mm inhibition zone for white radish water extract and ginger was found in recent research for *Lactobacillus agilis*. Garlic has a substantial inhibitory impact on *Lactobacillus agilis* but a lesser (10 mm) zone of inhibition compared to green chili, which has the biggest zone of inhibition (10.8 mm) against the same bacterium. These veggies have a strong antimicrobial impact, according to this study.

Keywords: Antimicrobial activity, Phytochemical, Gut microbes, *Lactobacillus agilis*, Phenolic compounds

Introduction

The so-called gut microbiota, found in the gastrointestinal compartment, is thought to consist of 10 to 100 trillion microorganisms, the majority of which are bacteria. Its size is estimated to be between 250 and 400 m^2. The human gut microbiota is thought to contain between 500 and 1000 different species of bacteria, in addition to an undetermined number of other microorganisms (Eisenstein *et al*., 2020) [5]. The host normally benefits from the gut microbiota's engagement in a variety of processes including hormone regulation, vitamin and amino acid production, proliferation of epithelial cells, protection from infections, and glucose and lipid metabolism (Lin *et al*., 2021) [9]. Dietary fiber is a crucial nutrient for maintaining the diversity of the gut microbiota. Although dietary fibers can be found in a wide variety of plant-based foods such as cereals, legumes, nuts, tubers, vegetables, and fruits, fiber intake in Western nations is much lower than the advised levels (Mobley *et al*., 2014) [10]. Our awareness of the significance of gut microbiota has substantially expanded in light of recent scientific discoveries. The appropriate growth of several physiologic elements, including the immunological and endocrine systems, depends on bacterial colonization of the gut. According to new research, gut bacteria may also affect how the host's brain development is regulated. Additionally, both the peripheral and central adult brain functions can be modified by the general composition of the microbiota as well as the effect of key species that cause particular responses. Due to the complex activity of their microbial communities, which can provide protective, immunologic, and metabolic functions as a result of active mutualistic associations between the microbial and host species, the gut microbiota is considered to be a very energetic metabolic organ (Holscher *et al*., 2017) [6]. Gut microbiota may be changed by adopting healthy dietary practices, which include consuming fruits and vegetables. The majority of people on Earth now consume Western diets, which are characterized by excessive consumption of saturated and omega-6 fatty acids and decreased consumption of omega-3 fatty acids, fruits, vegetables, and fiber (Myles *et al*., 2014) [11]. According to (Roses *et al*., 2021) [13], a sedentary lifestyle coupled with a Western-style diet can cause several inflammatory-related conditions, including metabolic syndrome, cardiovascular disease, and neurodegenerative diseases. The majority of these illnesses have been linked to changes in the human microbiota, particularly in individuals with lower bacterial richness and diversity (Le *et al*., 2013). Nearly 90% of the whole gut microbiota is made up of the phyla Firmicutes and Bacteroidetes, two dominating phyla. The ratio of these two prominent phyla can change as a result of several internal and extrinsic variables, but it typically stays the same in the majority of healthy organisms (Cardoneanu *et al*., 2021) [3]. Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia, and a few species from the Archaea domain are other phyla that contribute to the makeup of the host gut microbiota. In addition, the most significant genera of host gut microbiota that have been identified include *Lactobacillus*, *Escherichia*, *Bifidobacterium*, *Clostridium*, *Streptococcus*, and *Ruminococcus* (Backhed *et al*., 2005). It is believed that the human microbiota is a vast network that affects human health in innumerable ways. The complex functional role of gut microbiota can be gradually unearthed with the aid of contemporary technological advancements (Chen *et al*., 2021) [4]. Due to their high vitamin, mineral, phytochemical, and dietary fiber content, vegetables are essential for human health. Particularly essential functions in maintaining human health are played by dietary fiber and antioxidant vitamins (vitamins A, C, and E).

Materials and Methods

Sample preparation

All selected vegetables (ginger, garlic, onion, raw papaya, green chili, white radish) were purchased from the local market in Bijnor, India (U.P.), cleaned to remove soil, peeled off, and washed again under running tap water. Each of these vegetables was washed and then dried for two to four hours in the sun after being chopped into little pieces. Even if the food has been peeled, drying the whole item will still take at least 16 to 18 hours. This procedure takes 10 to 15 days. To be used in upcoming studies and extractions, all consumables should be produced as powder and stored at room temperature in sterile jars.

Qualitative phytochemical analysis of vegetables

Using the AOAS methodology (1990), as defined by Singh and Garg (2022) [16] and Trease and Evans (1978) [18] qualitative standard chemical tests were conducted for a phytochemical screening of vegetables. These tests all detected alkaloids, saponins, tannins, flavonoids, anthraquinones, terpenoids, and glycosides.

Antimicrobial activity by agar well diffusion method

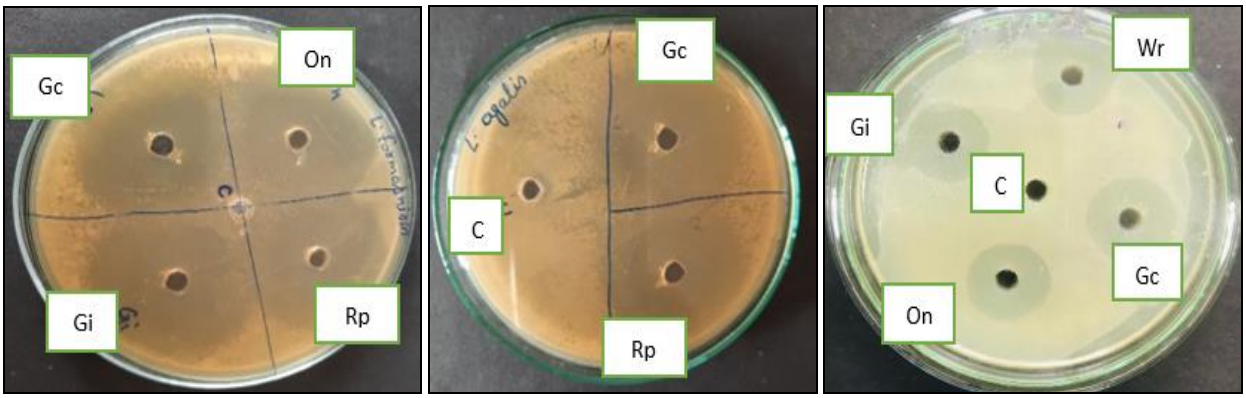
The antimicrobial activity spectra of five selected isolates, against gut microbes *Lactobacillus agilis, Bifidobacterium dentium, Lactobacillus buchneri, Lactobacillus formosensia, Aerococcus suis,* were examined by agar well diffusion assay. From Nutrient broth 100 µl of 24 h old cultures of the pathogenic bacteria were swabbed on Muller- Hinton agar (MHA) plates and afterward wells were made using a sterile cork borer. Wells were filled with 20µl of the supernatant of each extract and plates were incubated at 37℃ for 24 to 48 h. The diameter of the inhibition zones was measured and the mean diameter for the inhibition zone was calculated. The benefit of the well diffusion assay is that, as opposed to loading a disc, we can typically put more samples into a well at once. The phytocompound's ability to diffuse across the agar is crucial when employing the well diffusion method. A ruler, a set of calipers, or a template are used to measure the zone of inhibition. Its dimensions are expressed in millimeters and are typically rounded to the next millimeter. Also provided is the well's diameter. Without using any tools, these measurements are made with the naked eye.

Result and Discussion

Most vegetables include the majority of phytochemicals such as alkaloids, flavonoids, saponins, tannins, glycosides, sugars, and phenolic compounds. According to Table 1, ginger, garlic, onion, raw papaya, green chili, and white radish include alkaloids, flavonoids, glycosides, carbohydrates, and phenolic compounds. They exhibit anti-inflammatory, antifungal, antibacterial, antimycotic, and anticancer activities, according to Sami *et al* (2021) [14], who found these compounds in natural products such as plants. The study reveals that these vegetables also contain saponins, albeit they are not discovered in green chili, and terpenoids are not present in raw papaya, and onion while being present in these other vegetables (Table 1). Fruits and vegetables are the most abundant sources of phytochemicals. In addition to being used in human meals, phytochemicals are used as natural antibacterial agents in food preservation. The majority of its health benefits are due to its powerful antioxidant activity. Phytochemicals have a considerable influence on the regulation and prevention of naturally degrading processes as well as the growth of microorganisms, including diseases that affect food quality. Phytochemicals might be referred to as "plant chemicals." They are non-food chemical substances present in plants that have a multitude of good health impacts as well as disease-prevention capacities (Ahmed *et al*., 2014) [1]. Secondary metabolites are generally gathered by all plant cells, although concentrations vary depending on the portion of the plant, the seasons, the environment, and the many growth phases. Leaves are a great source of accumulation and are also quite useful (Jain *et al*., 2012) [7]. A new study underlined the important role that early gut microorganisms have in long-term human health. As a result, pregnancy-related factors such as gestational age, delivery technique, birth weight, feeding patterns, antibiotic exposure, and the maternal microbiome affect the composition of the early-life gut microbiota (Sarkar *et al*., 2012) [15]. Ginger and white radish have a 9 mm zone of inhibition against *Lactobacillus agilis*, according to the most recent data in Table 2. Garlic has a slightly smaller (10 mm) zone of inhibition than green chili (10.8 mm), which has the highest zone of inhibition against the same bacterium *Lactobacillus agilis* (Table 2). According to the data (Table 2; Fig.2), raw papaya has an inhibition zone of 9.8 mm against *Lactobacillus agilis*, while onion has an inhibition zone of 8.2 mm. The zones of inhibition for the same bacteria against raw papaya and white radish, on the other hand, are 9.8 mm and 9 mm, respectively (Table 2). Ginger and green chili show the same zone of inhibition (10.8 mm) for *Bifidobacterium dentium* bacterium. The inhibitory zones of garlic and onions against *Bifidobacterium dentium* are 9.6 mm and 9.8 mm, respectively, according to the results of the analysis (Table 2).

According to the data, ginger has a 10.2 mm zone of inhibition against the same bacteria *Lactobacillus buchneri*, while onion and green chili both have the largest zone of inhibition (10.9 mm) against this bacterium (Table 2). In comparison to garlic and white radish, which both have a 9.8 zone of inhibition against *Lactobacillus buchneri* (Table 2; Fig 2), raw papaya has a 10 mm zone of inhibition. When raw papaya and ginger, with zones of inhibition of 10 mm against *Lactobacillus formosensia*, are compared, white radish exhibits a slightly wider zone of inhibition (10.8 mm). *Aerococcus suis* and *Lactobacillus formosensia* are two distinct bacteria that garlic kills in the zone of 9.8 mm and 9 mm (Table 2). Garlic, on the other hand, has a 9.8 mm zone of inhibition against *Aerococcus suis*, while both green chili and garlic have a 9.8mm and 9 mm zone of inhibition respectively against *Lactobacillus formosensia* (Table 2; Fig. 2). However scientific research has demonstrated the therapeutic benefits of ginger, such as its anti-inflammatory and antioxidant characteristics, a more concentrated and unstudied bioactive is ginger's ability to protect neurons (Raul *et al*., 2022) [12]. One of the valuable medicinal plants found in Zingiber officinale is commonly used in food and pharmaceutical applications. Its crude extract is well-known for its pharmacological effects. The ginger rhizome, which is commonly used as a spice or nutritional supplement, has long been employed in traditional medicine (Swarupanada *et al*., 2022).

Fig 1: Different selected eatables for using water extracts



A- Lactobacillus formosensis ***B- Lactobacillus agilis*** ***C-Bifidobacterium. dentium***

Fig 2: Zone of inhibition of water extract of different eatables: green chili (G_c), raw papaya (R_p), onion (O_n), ginger (G_i), white radish (W_r), and raw papaya (Rp) and C denoted as water control against *Lactobacillus formosensis* plate **A**; *Lactobacillus agilis* plate **B**; and *Bifidobacterium. dentium* plate **C.**

Table 1: Phytochemical analysis of selected vegetables

Phytochemicals	Eatables of water extract					
	Ginger	Garlic	Onion	Raw papaya	Green chili	White radish
Alkaloids	+	+	+	+	+	+
Saponins	+	+	+	+	_	+
Tannins	_	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Anthraquinones	_	+	+	_	+	+
Terpenoids	+	+	_	_	+	+
Glycosides	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Phenolic compound	+	+	+	+	+	+

Table 2: Zone of inhibition of water extract of eatables after 24 hr. on gut microbes.

Bacterial strains	Eatables					
	Garlic(mm)	Ginger(mm)	Onion(mm)	Raw papaya(mm)	White radish(mm)	Green chilli(mm)
Lactobacillus agilis	10	9	8.2	9.8	9	10.8
Bifidobacterium dentium	9.6	10.8	9.8	10.2	10	10.8
Lactobacillus buchneri	9.8	10.2	10.9	10	9.8	10.9
Lactobacillus formosensia	9	10.0	10.9	10	10.8	9.8
Aerococcus suis	9.8	10	9	8	9.2	8.9

Conclusion

The results of our study revealed that extracts of selected vegetables such as ginger, garlic, onion, raw papaya, white radish, and green chili contain a variety of chemical elements such as phenols, proteins, carbohydrates, alkaloids, and saponins, supporting the assertion that the selected vegetables have biological properties such as antibacterial activity. Because these veggies are commonly available and the extract can be easily made using centrifugation, it could be a viable and less expensive alternative to existing pharmaceuticals. Natural goods or products derived from them are becoming increasingly popular in treating and preventing disease due to their decreased risk of harmful consequences. Vegetables are important to a healthy diet and significantly impact gut bacteria. Vegetables can potentially prevent and improve metabolic syndrome and other related disorders by regulating gut flora.

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Conflict of Interests: The authors state they have no relevant conflicts of interest.

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