



## Mini Review Article

## Pathogenic bacteria and parasites present in edible leafy greens

Ankush Kabiraj<sup>1</sup>, Sayan Bhattacharyya<sup>2,\*</sup>, Shibani Datta<sup>3</sup><sup>1</sup>All India Institute of Hygiene and Public Health, Kolkata, West Bengal, India<sup>2</sup>Dept. of Microbiology, All India Institute of Hygiene and Public Health, Kolkata, West Bengal, India<sup>3</sup>Dept. of Biochemistry, All India Institute of Hygiene and Public Health, Kolkata, West Bengal, India

## ARTICLE INFO

## Article history:

Received 06-06-2023

Accepted 26-06-2023

Available online 24-07-2023

## Keywords:

Tulsi

Mint

Spinach

Bacteria

## ABSTRACT

Tulsi, mint and spinach, all are examples of culinary herbs and beneficial for health. They have digestive, antibacterial and many other medicinal properties. Many people, however, have also studied and found presence of enteropathogens in such herbs and leafy greens. In the laboratory we have gone through different tests to identify the specific pathogen present on the leaves. This will be a milestone of public health research in food microbiology.

This is an Open Access (OA) journal, and articles are distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License](https://creativecommons.org/licenses/by-nc-sa/4.0/), which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: [reprint@ipinnovative.com](mailto:reprint@ipinnovative.com)

## 1. Introduction

Tulsi, mint and spinach all are herbs and very important as well as very beneficial for overall health and well-being. However, sometimes they contain some harmful bacteria and parasites that may cause food borne infections. Tulsi is sometimes used as divine offering or Prasad. Not many studies have tried to find out the load of pathogenic bacteria, parasitic cysts and ova causing foodborne infections, in these leafy vegetables. In this study we will try to find the pathogenic microorganisms present on these herbs and their detrimental effects on the health of consumers.

## 2. Tulsi

Holy basil (*Ocimum sanctum*) is commonly known as *Tulasi* in Sanskrit or *Tulsi* in Hindi.<sup>1</sup> Holy basil is a perennial flowering plant from the family called *Lamiaceae*. While this plant is native to the Indian subcontinent, it may also grow throughout Southeast Asia.<sup>2</sup> Tulsi has a special place in Ayurveda as well as the homes of Hindus in India. It

is considered sacred by Hindus and worshipped by them and is also used medicinally to relieve symptoms like cough and cold. Three main types of Tulsi are seen growing in India:

Bright green leaves called Ram Tulsi. Purplish green leaves called Krishna Tulsi. Common wild variants are called Vana Tulsi.

Tulsi has used as prasad or offering to God and also proved to be highly effective in protecting our body from various infections and diseases of the liver, skin, kidney, respiratory tract and other sites.

Tulsi is known to have many medicinal properties like:

1. It might be an antipyretic agent
2. It might have anti-inflammatory activity
3. It might have an antiemetic action
4. It might help lower blood sugar (antidiabetic effect)
5. It might act as a hypotensive ( can lower blood pressure)
6. It might have hypolipidemic activity
7. It might act as an analgesic

It might have anti-asthmatic activity

It might be an hepatoprotective (liver-protective) agent

\* Corresponding author.

E-mail address: [sayantheboss@yahoo.co.in](mailto:sayantheboss@yahoo.co.in) (S. Bhattacharyya).

- It might help reduce stress (antistress)
- It might be a potent expectorant (expels mucous)
- It might have anticancer potential
- It might be a diaphoretic (induces sweating).

Owing to its numerous health benefits, Tulsi is rightly called the 'Queen of Herbs'.<sup>3</sup>

In spite of having several beneficial roles, Tulsi may contain several harmful bacteria like- Enterobacteriales (a previous large family of Gram-negative bacteria that includes a number of pathogens such as *Escherichia coli*, *Klebsiella spp.*, *Enterobacter spp.*, *Citrobacter spp.*, *Salmonella spp.*, *Shigella spp.*, *Proteus spp.*, *Serratia spp.* and other species), *Pseudomonas spp.*<sup>4</sup> and coccidian parasites like- *Cyclospora spp.*<sup>5</sup>

### 3. Pudina

Mint, scientifically known as *Mentha spicata*, is an aromatic herb belonging to the family Lamiaceae. It is called Spearmint in English and Pudina in Hindi. It is widely used in Indian cuisines for its characteristic flavour. The herb originated in Europe.<sup>6</sup> Fresh and dried leaves and essential oils derived from Pudina are widely used in the food, cosmetic, confectionery, chewing gum, toothpaste, and pharmaceutical industries.<sup>7</sup> It is grown worldwide for commercial uses as a flavouring agent for food and to be used in medicines.<sup>8</sup>

The properties of Pudina are given below:

1. It may act as carminative (for removal of gas from the stomach)
2. It may show antispasmodic property (used to relieve muscle spasms)
3. It may show diuretic activity (causes the kidneys to make more urine)
4. It may show antibacterial and antifungal effects.
5. It may show antioxidant agent and minimize the damage caused by oxidants.<sup>7</sup>
6. It may show anti-diarrhoeal effects and also potent anti-asthmatic activity.
7. It may help to reduce cold & fever.
8. It may help to manage weight
9. It may act as an antidote to counteract some particular poisons.<sup>8</sup>

There is still no information about the presence of any pathogenic bacteria and parasites on or in pudina leaves, and people are searching for it.

### 4. Spinach

Spinach (*Spinacia oleracea*) is a green leafy flowering plant native to central and western Asia. It belongs to the family Amaranthaceae. It is consumed either fresh, after cooking, or after storage using preservation techniques by canning, freezing or dehydration. It may be

eaten cooked or raw, and the taste differs considerably. The high oxalate content may be reduced by steaming.<sup>9</sup>

The beneficial properties of spinach are as follows:-

1. Promotes weight management (reduce weight gain risk by up to 82%)<sup>10</sup>
2. Reduces cancer risk<sup>11</sup>
3. Protects eye health (rich in Vitamin A & C which helps to prevent age related macular degeneration)<sup>12,13</sup>
4. Prevents hair loss, being rich in iron<sup>14</sup>
5. Prevention of anaemia (loaded in Vitamin K & iron)<sup>15–17</sup>

#### 4.1. High in antioxidants, and scavenges free radicals and prevents oxidative stress<sup>18</sup>

Although spinach is quite beneficial for our health, a variety of bacteria can be present in it, like *Salmonella spp.* and *Shigella spp.*<sup>19–21</sup> Even parasites like- *Ascaris lumbricoides* eggs, *Entamoeba histolytica*, *Giardia lamblia* cysts may be present in it.<sup>22–25</sup>

Tulsi, pudina and spinach are all used as food as well as for medicinal purposes. Leafy vegetables like spinach, lettuce and pudina are good sources of dietary fiber and vitamins like Vitamin K. Tulsi (*Ocimum sanctum*) is also used as food rarely and also commonly as offering to God in temples for Prasad. Leafy vegetables have many antimicrobial properties and are also good source Vitamin K, Dietary fibers, mineral and Vitamin K. However, they can also contain many pathogenic microorganisms which can infect us by feco-oral route. Many researchers have been able to detect parasitic eggs and cysts along with pathogenic bacteria in leafy vegetables. High concentrations of thermotolerant coliforms (ThC), intestinal helminth eggs, and protozoa have been found in a wastewater-fed lake where water spinach was grown. Water spinach samples can contain *Cyclospora* (8%), *Giardia* cysts (56%), *Cryptosporidium* (17%).<sup>26</sup> Many workers have detected *Cyclospora* in vegetables in the local agricultural markets in the Central Valley of Costa Rica. The highest proportion of faecal coliforms was identified during the rainy season. *Cyclospora spp.* have been identified only in lettuce during the dry season.<sup>27</sup>

In other studies, protozoa *Giardia duodenalis* cysts have been found on lettuce.<sup>27</sup> In fact, fresh produce, in particular, as it is eaten with minimal preparation, is a very strong vehicle for transmission of bacterial and parasitic infections, and *G. duodenalis* cysts have been detected in such produce in many countries.<sup>27</sup>

VTEC (Verotoxigenic *Escherichia coli*) strain, *E. coli* O157:H7 'Sakai,' has also been found to colonize the roots and leaves of four leafy vegetables: spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa*), vining green pea (*Pisum sativum*), and prickly lettuce (*Lactuca serriola*).<sup>28</sup> This contamination is related to irrigation pattern, use of portable

toilets and the field worker personal hygiene. various factors like contaminated soil, fertilizer (manure/compost), wildlife, and irrigation water also contribute to presence of *Escherichia coli* in produce.<sup>29</sup> This can be a dangerous pathogen as it may also cause Hemolytic uremic syndrome (HUS). High burden of *Salmonella* contamination has been seen in leafy vegetables, including mint leaves, parsley and lettuce.<sup>30</sup> Moreover these leaves are consumed raw or by making sauce (e.g. Mint leaves).

Spinach leaf has also been found to contain harmful oocysts of coccidia like *Cryptosporidium parvum*.<sup>31</sup> Many foodborne illnesses in the United States are caused by germs on vegetables and fruits that people eat raw. Harmful germs sometimes found on leafy greens include *E. coli*, norovirus, *Salmonella*, *Listeria*, and *Cyclospora*.<sup>32</sup>

So keeping these things in mind, it can be surmised that are lots of pathogenic bacteria and parasites found in these leaves which can infect us.

In a study, fifty-eight leaf samples (58) were collected by us from the markets of different zones in Kolkata (east, west, north & south) and also other places of West Bengal.

Samples were collected in sterile universal container or if packed, then in packet. Then samples were transported to the laboratory for testing. Samples will be weighed, and specific weight or volume were cultured on the following media —

1. Mac Conkey agar with neutral red as pH indicator (Peptone, Neutral red, agar, Lactose, Sodium taurocholate, deionized water) for culturing aerobic LF or NLF bacteria.
2. Xylose Lysine Deoxycholate (XLD) media {Agar, Sucrose, Lactose, Sodium thiosulphate, L-Lysine, NaCl, xylose, Yeast extract, Sodium deoxycholate, Ferric ammonium citrate} for culturing aerobic bacteria like *Shigella* and *Salmonella* spp.
3. Robertson's cooked meat medium (RCM) {Cooked meat medium, Peptic digest of animal tissue, Dextrose, Sodium chloride, yeast extract, Iron filings, Hemin, Vitamin K} for culturing anaerobic bacteria. RCM were incubated for 2 days and then Gram stain was done to find anaerobic bacteria.
4. Selenite F broth for enrichment and later after 1 day of incubation at 37 degree C, 1 loopful was subcultured from it on to XLD agar and MacConkey agar again. This will be done to find *Shigella* spp. and *Salmonella* spp.
5. Normal saline and Lugol's Iodine were used to detect the trophozoites and cysts of protozoa, and egg & larvae of helminths by making wet mount.

One gram of dry sample was mixed in 1 ml normal (0.9%) saline in a sterile universal container, vortexed and transferred to 10 ml test tube. Then it was centrifuged at 5000 rpm for 2 minutes, and with deposit, saline

mount, Lugol's Iodine mount and Gram stain were done. Modified ZN stain was carried out with 4% sulphuric acid as decolorizer. Also culture on the aforesaid media were performed from the deposit. The colonies of the bacteria that appeared on the culture media after overnight incubation, were identified by Gram stain, Catalase, Oxidase and other standard biochemical tests.

We studied 58 samples. Among them 27 samples were of Tulsi, 18 were Mint and 13 were Spinach.

Cryptosporidium oocysts were present in 2 spinach samples.

Commonest bacteria in the leaves were:

1. **In Tulsi-** *Klebsiella aerogenes*, *Enterobacter cloacae*, *K. pneumoniae* and *Bacillus cereus*.
2. **In Pudina-** *E. cloacae*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa* and *Klebsiella oxytoca*.
3. **In Spinach-** *Aeromonas schuberti*, *K. aerogenes*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and Non-EHEC 5157:H7 *Escherichia coli*.

*Clostridium* spp. grew on 2 tulsi leaves on RCM.

Most leaf washings had pH 6. Figure 1 below shows image of tulsi leaf.



Fig. 1: Tulsi leaf

## 5. Discussion

Hence researchers have seen that leaves like Tulsi, Pudina and spinach do contain harmful bacteria and parasites which can cause gastroenteritis in the consumers. Protozoan parasites like *Giardia duodenalis*, *Cryptosporidium parvum* and *Cyclospora cayentanensis* have led to such vegetable or fruit-borne outbreaks of gastroenteritis. We also saw that

edible leaves like Tulsi and Pudina did harbour pathogenic microbes like *Bacillus cereus* and *Staphylococcus aureus*. Even *Clostridium* spp. were present. Such spore bearing bacteria may not be killed even by cooking and may cause gut infection. Edible leaves can thus harbour many pathogens which colonize them, and hence one should be very cautious while consuming them. These pathogens come on the leaves mainly from soil, water and air. These are somewhat neglected areas of public health and nutritional science. More studies are needed in these aspects.

## 6. Conclusion

The burden of gut pathogens in herbs and leafy greens may have possible health impact and should be the topic of future biomedical research.

## 7. Source of Funding

None.

## 8. Conflict of Interest


None.

## References

- Jamshidi N, Cohen MM. The clinical efficacy and safety of tulsi in humans: a systematic review of the literature. *Evid Based Complement Alternat Med*. 2017;2017:9217567. doi:10.1155/2017/9217567.
- Petruzzello M, Encyclopedia Britannica. Holy basil; 2019. Available from: <https://www.britannica.com/plant/holy-basil>.
- Garg P. Tulsi (Holy Basil): Health Benefits, Uses and Nutritional Value; 2023. Available from: <https://pharmeasy.in/blog/health-benefits-of-tulsi/>.
- UMass Extension Greenhouse Crops and Floriculture Program. Available from: <https://ag.umass.edu/greenhouse-floriculture/photos/basil-pseudomonas>.
- Friedman LF. Basil From Mexico Is Likely Cause of Cyclospora Food Poisoning Outbreak; 2019. Available from: <https://www.consumerreports.org/food-safety/basil-from-mexico-is-likely-cause-of-cyclospora-food-poisoning-outbreak/>.
- Available from: <https://plants.ces.ncsu.edu/plants/mentha-spicata/>.
- Snoussi M, Noumi E, Trabelsi N, Flamini G, Papetti A, DeFeo V. Mentha spicata Essential Oil: Chemical Composition, Antioxidant and Antibacterial Activities against Planktonic and Biofilm Cultures of Vibrio spp. Strains. *Molecules*. 2015;20(8):14402–24.
- Mahendran G, Verma SK, Rahman LU. The traditional uses, phytochemistry and pharmacology of spearmint (*Mentha spicata* L.): A review. *J Ethnopharmacol*. 2021;278:114266. doi:10.1016/j.jep.2021.114266.
- Spinach. Available from: <https://en.m.wikipedia.org/wiki/Spinach>.
- Nour M, Lutze SA, Grech A, Allman-Farinelli M. The Relationship between Vegetable Intake and Weight Outcomes: A Systematic Review of Cohort Studies. *Nutrients*. 2018;10(11):1626.
- Vaňková K, Marková I, Jašpřová J, Dvořák A, Subhanová I, Zelenka J, et al. Chlorophyll-mediated changes in the redox status of pancreatic cancer cells are associated with its anticancer effects. *Oxid Med Cell Longev*. 2018;2018:4069167.
- Vitamin A and Carotenoids: Fact Sheet for Health Professionals. Available from: <https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/>.
- Age-Related Macular Degeneration (AMD). Available from: <https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/age-related-macular-degeneration>.
- Park SY, Na SY, Kim JH, Cho S, Lee JH. Iron plays a certain role in patterned hair loss. *J Korean Med Sci*. 2013;28(6):934–8.
- Bai X, Long J, He X, Yan J, Chen X, Tan Y, et al. Overexpression of spinach non-symbiotic hemoglobin in Arabidopsis resulted in decreased NO content and lowered nitrate and other abiotic stresses tolerance. *Sci Rep*. 2016;6:26400. doi:10.1038/srep26400.
- Iron: Fact sheet for health professionals. National Institutes of Health Office of Dietary Supplements. Available from: <https://ods.od.nih.gov/factsheets/Iron-HealthProfessional/>.
- Vitamin K: Fact sheet for health professionals. National Institutes of Health Office of Dietary Supplements. Available from: <https://ods.od.nih.gov/factsheets/VitaminK-HealthProfessional/>.
- Pizzino G, Irrera N, Cucinotta M. Oxidative stress: harms and benefits for human health. *Oxid Med Cell Longev*. 2017;2017:8416763.
- Abadias M, Usall J, Anguera M, Solsona C, Vinas I. Microbiological quality of fresh minimally processed fruit and vegetables, and sprouts from retail establishments. *Int J Food Microbiol*. 2008;123(1-2):121–9.
- Oliveira MA, Oliveira VM, Bergamini AMM, DeMartini E. Microbiological quality of ready-to-eat minimally processed vegetables consumed in Brazil. *Food Control*. 2011;22(8):1400–3.
- Tambekar DH, Mundhada RH. Bacteriological Quality of Salad Vegetables Sold in Amravati City (India). *J Biol Sci*. 2006;6(1):28–30.
- Al-Binali AM, Bello CS, El-Shewy K, Abdulla SE. The prevalence of parasites in commonly used leafy vegetables in South Western, Saudi Arabia. *Saudi Med J*. 2006;27(5):613–6.
- Daryani A, Ettehad GH, Sharif M, Ghorbani L, Ziaei H. Prevalence of intestinal parasites in vegetables consumed in Ardabil, Iran. *Food Control*. 2008;19(8):790–4.
- Abougrain AK, Nahaisi MH, Madi NS, Saied MM, Ghenghesh KS. Parasitological contamination in salad vegetables in Tripoli-Libya. *Food Control*. 2010;21(5):760–62.
- Fallah AA, Piralı-Kheirabadi K, Shirvani F, Saei-Dehkordi SS. Prevalence of parasitic contamination in vegetables used for raw consumption in Shahrekord, Iran: Influence of season and washing procedure. *Food Control*. 2012;25(2):617–20.
- Ortega YR, Sanchez R. Update on *Cyclospora cayentanensis*, a food-borne and waterborne parasite. *Clin Microbiol Rev*. 2010;23(1):218–34.
- Cook N, Nichols RA, Wilkinson N, Paton CA, Barker K, Smith HV. Development of a method for detection of *Giardia duodenalis* cysts on lettuce and for simultaneous analysis of salad products for the presence of *Giardia* cysts and *Cryptosporidium* oocysts. *Appl Environ Microbiol*. 2007;73(22):7388–91.
- Crozier L, Hedley PE, Morris J, Wagstaff C, Andrews SC, Toth I, et al. Whole-Transcriptome Analysis of Verocytotoxigenic *Escherichia coli* O157:H7 (Sakai) Suggests Plant-Species-Specific Metabolic Responses on Exposure to Spinach and Lettuce Extracts. *Front Microbiol*. 2016;7:1088. doi:10.3389/fmicb.2016.01088.
- Park S, Navratil S, Gregory A, Bauer A, Srinath I, Jun M, et al. Generic *Escherichia coli* contamination of spinach at the preharvest stage: effects of farm management and environmental factors. *Appl Environ Microbiol*. 2013;79(14):4347–58.
- Sarré FB, Dièye Y, Seck AM, Fall C, Dieng M. High Level of Salmonella Contamination of Leafy Vegetables Sold around the Niayes Zone of Senegal. *Horticulturae*. 2023;9(1):97.
- Macarasin D, Bauchan G, Fayer R. *Spinacia oleracea* L. Leaf Stomata Harboring *Cryptosporidium parvum* Oocysts: a Potential Threat to Food Safety. *Appl Environ Microbiol*. 2010;76(2):555–9.
- Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED). Available from: <https://www.cdc.gov/ncezid/dfwed/index.html>.

## Author biography

**Ankush Kabiraj**, 2nd Year Student

**Sayan Bhattacharyya**, Associate Professor  <https://orcid.org/0000-0001-7741-0866>

**Shibani Datta**, Director Professor and HOD

**Cite this article:** Kabiraj A, Bhattacharyya S, Datta S. Pathogenic bacteria and parasites present in edible leafy greens. *Indian J Microbiol Res* 2023;10(2):55-59.