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Evaluating the Antibacterial Efficacy of Traditional Indian Herbs on Escherichia coli

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Abstract
This study examines the antibacterial properties of various herbs commonly used in Indian cuisine, focusing specifically on their effects against Escherichia coli. The research investigates the effectiveness of Neem (Azadirachtaindica), Turmeric (Curcuma longa), Tulsi (Ocimum sanctum), Garlic (Allium sativum), and Lemon (Citrus limon) as natural antimicrobial agents. The methodology involves preparing herbal extracts and evaluating their antibacterial efficacy using the agar well diffusion method, with zones of inhibition measured to determine the extent of bacterial growth suppression. The results demonstrate significant antibacterial activity across all herbs, with Neem exhibiting the highest level of efficacy. The study contributes to the expanding evidence supporting the use of traditional medicinal plants in modern healthcare, emphasizing their importance in combating bacterial infections and enhancing public health.

Keywords: Antibacterial, herbal extracts, medicinal plants, Escherichia coli

Introduction

Medicinal plants serve a critical role in healthcare. The need for medicinal plants in health care is approximately 70-80 percent (Kunle and Oluwafemi, 2021). Several factors contribute to the growing acknowledgment of therapeutic plants, including their cultural acceptability, accessibility, affordability, and ability to address psychological demands (Sabina, and Karmen, 2024). The traditional use of low-profile and lesser-known medicinal plants should be documented in order to communicate their therapeutic efficacy, paving the path for the development of acceptable medication and reducing pressure on overexploited species. WHO acknowledged that medicinal plants play an essential role in the healthcare of around 80% of the world's population in underdeveloped nations, who rely heavily on traditional medicine (WHO, 2022). Food-borne illnesses have long been a serious problem in both poor and industrialized countries.

E. coli is the most common bacterium in the human digestive tract. Under normal conditions, its presence promotes digestive processes. However, when present in excess or in a virulent form, it causes sickness (Mueller and Tainter, 2023). Virulent forms of E. coli can cause gastroenteritis, urinary tract infections, newborn meningitis, and other complications (Pokharelet al., 2023). As a result, search for novel antimicrobial agents has become required. For millennia, a large proportion of India's population has relied on traditional remedies. The study of medicinal plants as a source of pharmacologically active chemicals has become more popular around the world. It is recognized that in developing countries like India, plants are the main medicinal source to treat infectious diseases. Since beginning of civilization, plants and their products are used as medicines.

The therapeutic usage of plants has even been recorded in 'Rigveda' between 4500 and 1600 B.C. (Rastogi and Mehrotra 2002). The study of therapeutic plants as natural products is becoming increasingly popular around the world (Gazzaneoet al., 2005). These plants contain active chemical components with strong antioxidant qualities that aid in disease prevention (Hakkimet al., 2008). Bacteria utilized as therapeutic or remedial agents have the genetic ability to transmit and acquire drug resistance (Cohen, 1992), therefore finding alternative treatments for bacterial illnesses is critical (Joao et al., 2004).

Antibiotic-resistant pathogenic bacteria have been identified as a significant threat to humans and animals by the World Organization for Animal Health (WHO 2023). Herbal materials are becoming more popular as alternatives to synthetic or chemical medications. Herbal ingredients are used in medicines. Herbs can be employed as plant extracts or active components. Furthermore, the majority of the world's population relied on herbal materials for their potent antibacterial qualities and primary healthcare benefits (Shokoh et al., 2020). The purpose of this study is to assess the antibacterial properties of different herbs against Escherichia coli. The following herbs, commonly used in traditional Indian herb preparations, Neem (Azadirachtaindica), Turmeric (Curcuma longa), Tulsi (Ocimum sanctum), Garlic (Allium sativum), Lemon (Citrus limon) were selected for the study to promote awareness of their utility.

Materials and method

Pure and analytical grade chemicals (Beef extract, peptone, Muller Hinton Agar sodium chloride) were used in media preparation for growth of bacterial culture. Bacterial Culture of Escherichia coli was used in this investigation. Culture of Escherichia coli NCDC249 was taken from National Collection of Dairy Culture (NDRI), Karnal, India.

Preparation of different herb extracts and inoculation in the media

Before being macerated with 70% ethanol in an Erlenmeyer flask, 50 gm of the powdered leaves were weighed on an analytical scale. Using a rotary shaker the powder was macerated entirely for nine days, with occasional shakes. Whatman No. 1 was used to filter the retrieved material. Extract filtrate was concentrated in a rotary evaporator at 50 °C and 50rotations per minute (rpm). Prepared herbal extract was put in bottles and refrigerate at 4 °C (Wasihun et al., 2023).

Revival of E. coli culture

Nutrient broth was inoculated with E. coli. Flasks were incubated for 48 hrs at 37°C in incubator. Inoculum was taken from incubated flasks in different dilutions and inoculated in plates containing nutrient agar with herbal extracts. Plates were inoculated at 37°C for 48 hrs in bacteriological incubator and the colonies were observed and counted using colony counter.

Antimicrobial activity

The antimicrobial activity was determined using the agar well diffusion method. The cultures were maintained at 4°C on Muller Hinton Agar. The bacterial cultures were inoculated in the Nutrient Agar plates. Then the herb extract was poured into well on all the plates with the help of micropipette (20µl/ml). The plates were then incubated at 37°C for 24 hrs and zone of inhibition was measured.

Result and Discussion

Antimicrobial efficiency of herbal extracts used in this report takes place using Agar well diffusion method. All herbal extracts showed antibacterial property against Escherichia coli bacterial. The results observed are shown in table 1 and figure 1 and2.

Table 1: Antibacterial efficiency of different herbal extracts

Sr. No	Common Name	Scientific Name	Zone of Inhibition in mm
1	Neem	Azadirachtaindica	28
2	Turmeric	Curcuma longa	20
3	Tulsi	Ocimum sanctum	15
4	Garlic	Allium sativum	14
5	Lemon	Citrus limon	22

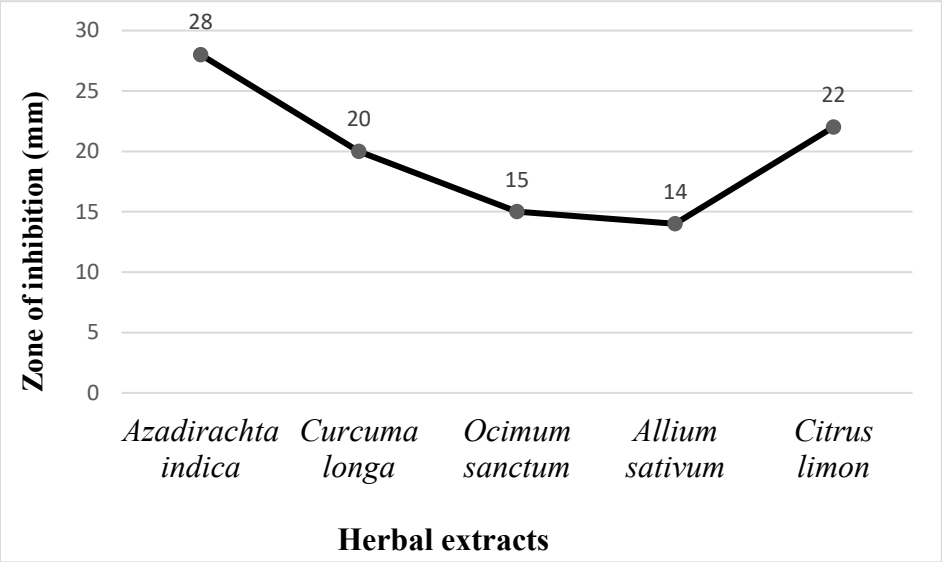

Figure 1: Graphical representation of antibacterial effect of different herbal extracts.

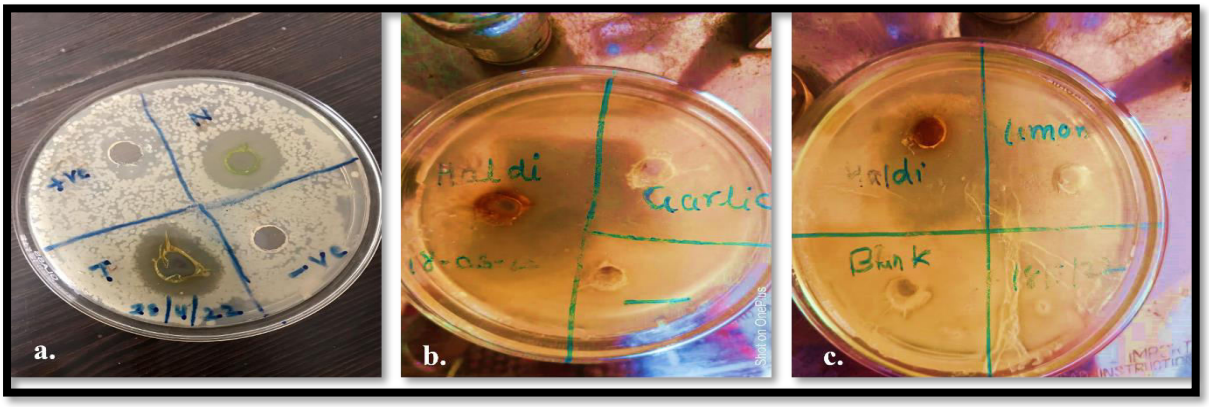

Figure 2: Antibacterial effect of herbal extracts shown by zone of inhibition, a. Neem and Tulsi extract (N, T), b. Turmeric (Haldi) and Garlic extract, c. Lemon extract and Turmeric (Haldi)

Our findings are consistent with those of Parham et al., (2020), who found that herbal medicines such as clove, portulaca, tribulus, eryngium, cinnamon, turmeric, ginger, thyme, pennyroyal, mint, fennel, chamomile, burdock, eucalyptus, primrose, lemon balm, mallows, and garlic can eliminate bacteria by acting as a free radical scavenger. These herbal medications have varying antibacterial action against different types of bacteria, as do their extracts and essential oils. In this light, it was demonstrated that the minimum inhibitory concentration (MIC) of these herbal medicines, such as thyme essential oil, against various bacteria is in the range of 50-400 ppm for E. coli,

S.aureus, P. mirabilis, S. typhimurium, P. vulgaris, Y. enterocolitica, S. marcescens, B. licheniformis, P. putida, S. flava, P. fluorescens, L. innocua, Micrococcus spp., and B. thuringiensis.

Our findings align with the research conducted by Oyas Ahmed Asimi et al., (2013), which also supported the beneficial properties of herbs and spices. Their study highlighted that assessing antioxidant activity often requires multiple methods. Their results confirmed both the antioxidant and antimicrobial properties of spice extracts. Since it's challenging to evaluate the antioxidant activity of spices using just one method, they employed three different approaches DPPH, FRAP, and TPC. The findings revealed that the spice extracts used in their study are rich in natural compounds with significant antioxidant potential. Regarding antibacterial properties, the spices were ranked in effectiveness as follows: cinnamon > cumin > ginger > garlic > turmeric. Their research also showed that the ethyl acetate extracts of these spices possess strong antibacterial and antioxidant effects, suggesting potential applications in aquaculture.

The findings are consistent with those of Gunasekara et al., (2017), who demonstrated that aqueous extracts of B. ceylanica showed inhibitory activity against isolates of Candida albicans. C. albicans is known to be a major pathogen responsible for human candidiasis. However, in recent years, non-albicans Candida species, such as Candida parapsilosis, Candida glabrata, Candida tropicalis, and Candida dubliniensis, have also been increasingly identified as both pathogens and commensals. Notably, the incidence of C. parapsilosis infections has risen significantly over the past few decades, making it the second most frequently isolated Candida species from blood cultures. The prophylactic and empirical use of antibiotics and antifungals plays a key role in the development of antimicrobial resistance among these pathogens.

Conclusion

The antibacterial activity of traditional Indian herbs - Neem, Turmeric, Tulsi, Garlic, and Lemon against Escherichia coli was assessed using the agar well diffusion method. The results suggest that all herbs have strong antibacterial activity, with Neem being the most effective, followed by Lemon, Turmeric, Tulsi, and Garlic.

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