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Research Article

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Effects of Neem leaves (*Azadirachta indica*) on food-borne pathogens

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Abstract

Over the years, people from all over the world have employed a variety of medicinal plants in daily life to treat illnesses. Human illnesses, particularly those that are food-borne, have been treated and controlled for a very long time using herbal medicine. It can also lessen any negative effects that can result from using pharmaceutical medications. However, using plants or herbal remedies may have adverse effects. Having insufficient knowledge about how herbs affect food-borne illnesses, their mode of action, and potential adverse effects is the main obstacle to using herbs as therapy. Neem (*Azadirachta indica*) leaf water extract has been shown to have antimicrobial activity against human pathogenic bacteria, including *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Shigella flexineri* at concentrations of 200 mg/ml, 150 mg/ml, 100 mg/ml, and 50 mg/ml. *E. coli* exhibits the maximum zone of inhibition at a dose of 200 mg/mL, whereas *Staphylococcus aureus* exhibits the lowest zone of inhibition, which is 9 mm, at a concentration of 50 mg/mL.

Keywords: Antimicrobial activity, Neem leaf, Herbal therapy, Medicinal plants, diffusion method.

Introduction

Azadirachta indica is thought to be indigenous to the Indian subcontinent, specifically the Indian area and Bangladesh, as well as Indochina's Cambodia, Laos, Myanmar, Thailand, and Vietnam. From South America to Indonesia, it has been widely adopted in other tropical and subtropical climates. A. Juss, 2020). When neem leaves are torn, they taste and smell distinctly grassy and are exceedingly bitter. White, fragrant blooms and fruit with a bittersweet pulp are also produced by neem trees in the form of yellow, olive-like fruit. The time Since the dawn of humanity, foodborne infections have seriously threatened all communities' health. These illnesses have undergone significant variation

throughout time, and the forms, severity, and effects of each greatly vary between different nations, regions, and groups. Foodborne illnesses are a significant contributor to morbidity and death throughout the globe as well as a significant barrier to socioeconomic growth. Because they serve as the gut bacteria's primary source of energy, nutrients play a crucial role in health. It is well known that eating the recommended quantity of veggies enhances both physical fitness and bodily processes. Numerous plants have antibiotic qualities that can treat bacterial illnesses. In South East Asia, one of the widely used medicinal herbs is neem (*Azadirachta indica*) (Murthy &Sexena, 1998). Before the discovery of microorganisms, people utilized plants to cure common infectious disorders, and the concept that some plants may be used as medicines was widely accepted (Rios and Recio, 2005). According to Edeoga *et al*. (2005), the chemical constituents in these plants are what give them their therapeutic value since they have a clear physiological effect on people or animals. It may also be found in many tropical and subtropical nations worldwide (Alzohairy, 2016). Because of their effective results in treating a variety of infectious and non-infectious chronic disorders, medications derived from the neem plant have saved the lives of several people from impoverished nations (Megalaa *et al*., 2018). The oral microbiota is also altered by these medications, which also cause sickness, diarrhoea, and tooth discoloration as unwanted side effects. Today's herbal medicines demonstrate their safety in usage as opposed to synthetic pharmaceuticals, which are viewed as being hazardous to both persons and the environment (Verma *et al*., 2020). There are hundreds of compounds (also known as phytochemicals) present in neem trees, many of which have been discovered to be bioactive and have a variety of uses on their own. Some of the most prevalent phytochemicals, such as azadirachtin, gedunin, and nimbolide, have already been discovered as possible medications with a variety of biological actions out of the more than 300 distinct substances that have been

found inside the neem tree (Nagini *et al*., 2021). Alkaloids, flavonoids, tannins, and phenolic compounds are the most significant of these bioactive ingredients, which are mostly secondary metabolites (Anyanwu and Nwosu, 2011). Cells of microorganisms are poisoned by these phytochemicals. According to Ratnasooriya *et al*. (2005), medicinal plants often include a variety of chemicals that have the potential to function as natural antibiotics in the treatment of common bacterial diseases.

Materials and Methods

Sample Collection

Neem leaves were collected from the field of Krishna College of Science and Technology Bijnor (U.P). The plant leaves were healthy and free from any deformities (Fig 1). The leaves were brought to the laboratory for further processing. Foodborne pathogens *E. coli (ATCC8739), Staphylococcus aureus (ATCC 6538), Shigella flexneri (ATCC 12022),* and *Pseudomonas aeruginosa (ATCC 9027)* will be obtained from IMTECH, Chandigarh.

Extract Preparation

Azadirachta indica leaf weights totaled 50 grams, which were then air-dried at room temperature in the shade (Fig 1). With the use of a blender, the leaves were divided into tiny bits and powdered. To get particles of the same size, the powder is subsequently passed through the sieve (Fig 2). The extract should be stored aseptically in an airtight container in an environment free of moisture. To satisfy the requirements for extractability and regulatory compliance, the solvents are carefully chosen for extraction. A conical flask containing 25mL of distilled water and 5 gm of powder is correctly weighed, transferred, shaken well, and properly mixed in water. The powder and water mixture are placed in an aseptic flask at room temperature for 7 to 8 days before being extracted and filtered using a muslin cloth and Whatman filter paper (Fig 3). The pure extract was obtained in the form of supernatant after centrifuging the liquid material

that had been filtered at 4,000 rpm for 5 min. For subsequent use, this pure extract was kept at 4 . Then, we use various amounts of extract for various dilutions to achieve various antibacterial activities. For example, we use 2 g of neem powder for 200 mL, 1.5 g for 150 mL, 1 g for 100 mL, and 0.5 g for 50 mL, and mix well to achieve various antimicrobial activities.

Physical analysis of *Azadirachta indica* (Neem) Description

Neem leaves range from medium to large in size and form from elongated to oblong, measuring around 20 to 40 centimeters on average. The shiny, smooth leaves have sharp, serrated edges and are a vivid green colour.

Moisture Contents

We were able to gauge the moisture levels by weighing a fresh sample of Neem leaves that had been shade-dried for 7 to 14 days.Neem leaves should be chosen and the leaves weighed once an hour during the period. The procedure should be repeated if any leaf is not completely dry. When the leaves are completely dry, make a powder, and then calculate the total final weight of the food. The predicted moisture was determined using the formula below.

Moisture content (%)

$$= \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Quantitative analysis of Neem leaves

Quantitative standard chemical tests were carried out for the phytochemical screening of shade-dried neem leaf samples using the AOAS approach (1990), as described by Trease and Evans (1978) and Singh and Garg (2022). Glycosides, alkaloids, saponin, tannin, flavonoids, and anthraquinones were all detected using these methods.

Antimicrobial activity of Neem leaves by agar well diffusion method

The agar well diffusion technique was used to examine the antibacterial activity of neem leaves. The chosen bacterial strains were added to 10 mL of Muller Hinton broth and then cultured for 24 hours at 37 . Separate 100 µL bacterial strain inoculations were applied uniformly and aseptically to the Muller-Hinton agar plates' whole surface. A sterile crock borer (6mm) was used to aseptically cut the well, and 50 µL of test extract was then added. The plates were exposed to a variety of food-borne pathogens for 24 to 48 hours while in a position at 37 .

Results and Discussion

By deducting the weight of the dried goods from the fresh weight, it was possible to calculate the amount of moisture that evaporated using the sundry approach. Neem leaves had a moisture content of 29.69%, as determined by the study of the neem leaves. To prevent fungi and microbes from growing, the extract's moisture content shouldn't be more than 30%. The neem leaf extract from Krishna College had a total ash concentration of 1.25%. Ash level should be of minimal importance because it denotes the presence of heavy metal contamination that is resistant to high temperatures. Neem leaf water-soluble extract content was 12.97% (Table 1).The antibacterial activity of neem leaf water extract was assessed against both negative bacteria in accordance with the findings on cultured Muller Hinton agar plates. Leaf extract of *Azadirachta indica* showed more inhibition zone against *Bacillus subtilis*, while *E. coli* and *S. typhi* are less susceptible to neem extract. All test organisms were significantly impacted by the aqueous extract of neem leaves. We evaluated the antibacterial effectiveness of various amounts of aqueous extracts from *A. indica* leaf against human pathogenic microorganisms. We discovered that leaf extract had significant antibacterial action against specific Gram-negative bacteria at the various quantities studied. According to M. Mustafa (2016) and R. Subapriya *et al*. (2005), neem offers a wide spectrum of

medicinal activities, including immune-stimulant activity, antipyretic, antioxidant, antiviral, and anti-inflammatory activity. Additionally, neem is used in medical toothpaste, locations, and soaps (H. Schmutterer, 2004). Neem also has a number of other uses, including being antibacterial, therapeutic, and anthelmintic. Table 2 presents the findings of the qualitative study on the neem plant (*Azadirachta indica*). The phytochemical test was carried out to determine the presence of chemically active components such as saponins, tannins, phenols, glycosides, terpenoids, flavonoids, alkaloids, and reducing sugar. These phytochemicals are widely employed in the drug and pharmaceutical industries and have been shown to have a variety of biological and therapeutic effects. According to the results, *Shigella flexineri* has the smallest zone of inhibition, with a zone of inhibition of 19 mm against a 200 mg/mL concentration (Table 3 and Fig. 4 E), while *E. coli* has the largest zone of inhibition, with a zone of inhibition of 24 mm, against a 200 mg/mL concentration. *Shigella flexneri*, *Staphylococcus aureus*, and *E. coli* all have a 16 mm zone of inhibition at the 150 mg/mL concentrations, while *E. coli* also has a 16 mm zone of inhibition at the 100 mg/mL concentrations. A 16.0 mm zone of inhibition is also present in the case of *E. coli* at 100 mg/mL concentrations, while a 12.5 mm zone is present at 50 concentrations. Neem includes compounds that may lower blood sugar levels, treat stomach ulcers, prevent pregnancy, eliminate bacteria, and stop plaque from accumulating in the mouth. Neem is used for a variety of ailments, although the majority of them lack solid scientific backing, including lice, tooth decay, gingivitis, psoriasis, and insect repellent. Additionally, there isn't any solid proof to back up the use of neem for COVID-19. As a pesticide, neem seed oil is employed.

Fig: 1 Neem leaf

Fig: 2 Powder

Fig: 3 Water extract

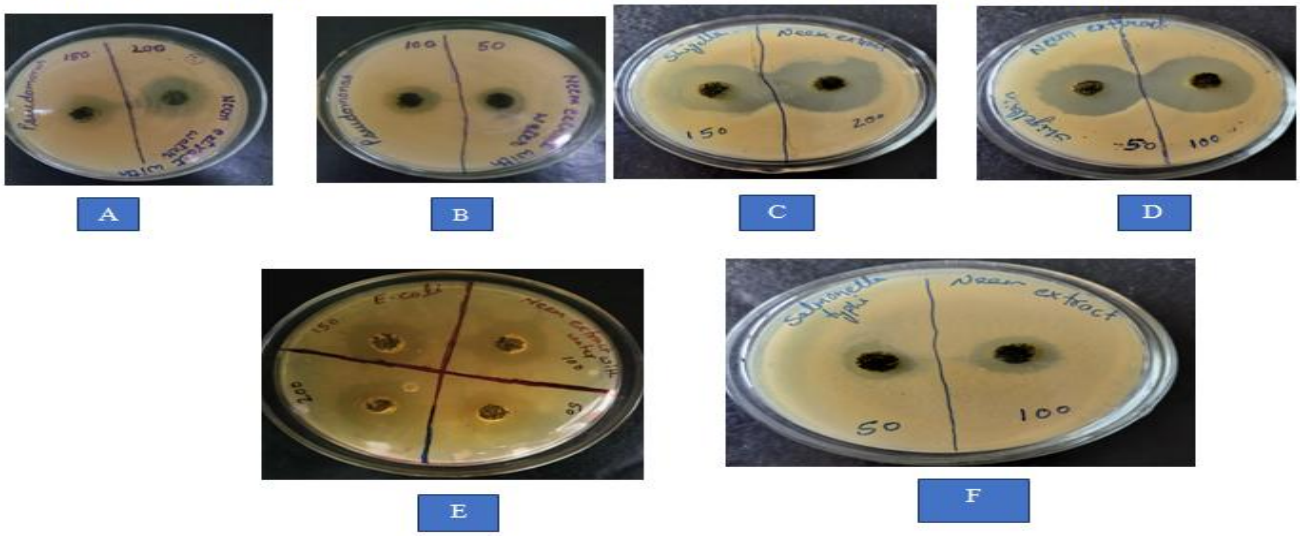

Fig 4: Antimicrobial activity of aqueous Neem extract at different concentrations against different food-borne pathogens. Plate A shows 200 and 150 concentrations against *Pseudomonas aeruginosa*; Plate B shows 100 and 50 concentrations against *Pseudomonas aeruginosa*. Plate C shows 200 and 150 concentrations against *Shigella flexineri*; Plate D shows 100 and 50 concentrations against *Shigella flexineri*; Plate E shows 200, 150, 100, and 50 concentrations against *E. coli* and Plate F shows 100 and 50 concentrations against *Salmonella typhi* respectively.

Table 1: Characteristics of Neem (%)

Characteristics of *Azadirachta india* (Neem leaf)	
Moisture content	29.67%
Total ash content	1.27%
Water soluble ash content	12.97%

Table 2: Qualitative analysis of *Azadirachta indica*

Phytochemicals	Water extract of (*Azadirachta indica* (Neem leaf)
Alkaloids	+++
Flavonoids	+
Saponins	++
Tannins	+
Terpenoids	+
Reducing sugar	++
Glycosides	+

Table 3: Zone of inhibition of neem leaves of water extract on different concentrations on selective Gram-negative bacteria after 24 hr.

Bacterial strains	Zone of inhibition of neem leaf water extract after 24 hr			
	200mg/ml	150mg/ml	100mg/ml	50mg/ml
Pseudomonas aeruginosa	20 mm	18 mm	14.5 mm	12 mm
Staphylococcus aureus	22 mm	16 mm	15.2 mm	9 mm
Shigella flexneri	19 mm	16 mm	14.25 mm	10.5 mm
E.coli	24 mm	19 mm	16.0 mm	12.5mm

Conclusion

Our study's findings revealed *Azadirachta indica* leaf extracts include a variety of chemical elements, including phenols, proteins, carbohydrates, alkaloids, and saponins, supporting the assertion that the plant exhibits biological properties including antibacterial activity. Since the plant is widely available and the extract is easily prepared using a straightforward maceration and centrifugation technique, it may be a genuine and more affordable alternative to traditional medications. A new hope for fending off the grave dangers posed by mounting evidence of antibiotic resistance is medicinal plants' antibacterial activity. Due to their lower risk of adverse effects, natural goods or products derived from them are becoming increasingly popular in treating and preventing disease. Since ancient times, people have employed neem and its components, especially in the Indian Subcontinent, for therapeutic purposes.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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