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IN-VITRO ANTIBACTERIAL ACTIVITY OF SELECTED INDIAN AROMATIC PLANTS AGAINST PATHOGENIC BACTERIA (SALMONELLA)

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ABSTRACT

This study aimed to treat the pathogenic activity of salmonella by plant extracts. Salmonella is an essential human pathogenic bacterium that causes various types of infections. Many plants have antimicrobial activities that can be used against the microbes. Salmonella was isolated from the sewage water and identified by different morphological and biochemical tests- Gram staining, sugar fermentation, catalase, MR, motility etc. Six plant samples Cardamom *(Elettaria Cardamomum),* Cloves *(Syzygium aromaticum),* Ginger *(Zingiber officinale),* Onion *(Allium sepa),* Turmeric *(Curcuma longa)* and Tulsi/holy basil *(Ocimum tenuiflorum)* were taken for antimicrobial activity against Salmonella. An antimicrobial susceptibility test was applied to see which plants were hyperactive to resist it. The mean value of the Zone of inhibition seen was Ginger-17.75mm, Onion-17.5mm, Cardamom-17mm, Turmeric-9mm, Tulsi-7mm and Clove-0mm respectively. The highest antimicrobial potential was observed for ginger and the lowest was for Tulsi. So Ginger, Cardamom and Onion can be used in the future to cure Salmonella infections.

KEYWORDS: Antimicrobial susceptibility, Plant extract, EMB, Biochemical test, Antibiotics.

INTRODUCTION

The World's leading cause of bacterial foodborne illness salmonella remains in first place. salmonella infection is causing an economic burden on both industrialized and

underdeveloped countries through the costs associated with surveillance, prevention and treatment of the disease.

It continues to be a significant global public health issue.[1] Salmonella, characterized as a rod-shaped, gram-negative facultative anaerobe, is a member of the Enterobacteriaceae family. around 2600 (within the genus Salmonella) serotypes have been identified with the use of the standard Kauffman – White scheme and most of these serotypes can adapt within a variety of animal hosts, including humans. most frequently isolated foodborne pathogens are salmonella and campylobacter, and are predominantly found in poultry, eggs and dairy products. Other food sources that are involved in the transmission of salmonella include fresh fruits and vegetables in general, food animals such as swine, poultry and cattle are the prime sources of salmonella infection.[2] In 1855 Theobald Smith first discovered and isolated salmonella from a pig's intestine that was infected with classical swine fever. On the name of Dr Daniel Almer Salmon, an American pathologist who worked with Smith the bacterial strain was named. The nomenclature of salmonella is controversial and still evolving. Currently, the nomenclature is used as recommended by the WHO (World Health Organisation) collaborating center.[3]

Salmonella causes different infectious diseases in humans and also in other animals. Many antibiotics and their chemical expressions are used to fight against them. distinct plants and their extracts are used against the harmful bacterial activity of salmonella. These plants have a history of healing treasures (medicinal value).[4] The antimicrobial activity of plants is researched by many researchers in the world, particularly in Latin America. A study tested 122 known plants utilized for cure in Argentina.[5]

Despite having generated a plethora of novel antibiotics over the past three decades, bacteria resistance to these medications has surged. Generally, bacteria possess the genetic capability to both transmit and acquire drug resistance, especially concerning therapeutic agents. This is a cause for alarm, particularly with a significant number of immunocompromised patients in hospitals and the emergence of multi-resistant bacterial strains. As a result, hospitals may experience new infections leading to increased mortality rates.[7] Plants have served as a valuable reservoir of natural substances for promoting human well-being over an extended period. This significance has been particularly emphasized in the past decade, marked by intensified investigations into natural therapies. The utilization of plant-derived compounds for pharmaceutical applications has seen a gradual rise in Brazil. According to the World

Health Organization,[8] medicinal plants are considered a prime source for a diverse range of drugs. Notably, approximately 80% of individuals in developed nations resort to traditional medicine, which often incorporates compounds sourced from medicinal plants. As a result, it is imperative to delve into the comprehensive exploration of these plants to gain a deeper understanding of their properties, safety, and efficacy.[9] The utilization of plant extracts and phytochemicals, both possessing well-documented antimicrobial properties, holds considerable significance in therapeutic applications. In recent years, numerous studies conducted in various countries have aimed to substantiate this efficacy.[10-16] Many plants have been selected for their antimicrobial attributes, stemming from compounds synthesized in the secondary metabolism of these plants. These active substances include phenolic compounds found in essential oils.[17] and tannins.[18]

A list of selected plants, chemical constituents present, and parts used to extract are (table No. 1) mentioned below.

Table 1: Phytochemicals of Plant Samples.

S.No.	Plant	Plant part	Chemical constituents	References
1	Cardamom *(Elettaria Cardamomum)*	Fruit	Phenolic acids, Flavonoids, Antioxidants, Monoterpenes	[19,20]
2	Ginger *(Zingiber officinale)*	Rhizome	Terpenes, Phenolic compounds	[21]
3	Tulsi *(Ocimum tenuiflorum)*	Leaves	Eugenol, Carvacrol, Flavonoids	[22]
4	Onion *(Allium sepa)*	Bulb	Flavanols, Organosulphur, Polyphenols, Ascorbic acids	[23]
5	Turmeric *(Curcuma longa)*	Rhizome powder	Polyphenolic compounds (Curcuminoids)	[24]
6	Clove *(Syzygium aromaticum)*	Dried buds	Eugenol, eugenol acetate, limonin, flavonoids, Thymol	[25]

MATERIALS AND METHODS

Chemicals and Reagents

Peptone, Agar, Sodium chloride, Hydrogen peroxide, Methyl red, Phenol red, Yeast, Sucrose, Dextrose, D- mannitol, Urea, Lactose, Kovac's reagent, Potassium dihydrogen phosphate, Eosin, Dipotassium phosphate, Methylene blue, Saffranine, Crystal violet, Ethanol, Tryptone.

Apparatus

Autoclave, Laminar air flow, Incubator, Vortex, Test tubes, Spreader, Inoculating loop, Sprit lamp, Eppendorfs, Refrigerator.

Isolation and Identification of Salmonella

Sample collection

The sample was collected from the nearby sewage of our lab (Sector 10-Noida, UP, India) during September Month. It contains wastewater from houses and roads.

Bacteria is confirmed by the colonial appearance on the EMB plate and following biochemical tests.

Isolation

The collected sample was spread over an EMB plate. After incubation of 24 hours at 37℃ various colonies appeared and then bacteria were inoculated in nutrient broth.

Identification

Identification of bacteria was done by biochemical tests, Explained below.

Biochemical tests

Gram staining- A smear for every isolated bacterium was prepared on a clean glass slide by heat fixing. Crystal violet was poured for 1 minute on it and cleaned with water. The smear was flooded with iodine solution. To decolorize it was washed with 95% ethanol and cleaned with water. After drying the glass slide under the microscope, we look for the bacteria.[6]

Catalase test- 3% hydrogen peroxide was dropped 20 µL on a clean glass slide and 20 µL bacterial broth was added to it. Observation of air bubble formation persisted for 40-50 seconds.[6]

Motility and Flagellate test- The assessment of motility involved employing the hanging drop technique to categorize the isolates as either motile or non-motile species. Initially, a minute droplet of MRS broth containing a specific isolate was deposited at the center of a sanitized cover glass. Following this, a concave depression slide was affixed to the cover glass using Vaseline, with the slide's recess facing downward onto the broth droplet. Subsequently, the inverted slide was examined in duplicate under the microscope at 40X and 100X respectively.[26]

Urease Test- The urease test aids in the recognition of microorganisms possessing the capability to produce the urease enzyme, which falls under the amidohydrolases and phosphodoesterases superfamilies. Urease facilitates the hydrolysis of urea into NH3 and

carbon dioxide. This enzymatic activity results in a shift in the medium's pH to alkaline, with a concomitant color change to pink at pH 8.1, indicating positive results. The test serves as a means to identify Helicobacter pylori, a urease-positive bacterium. To conduct the test, mucosa from an infected stomach or bacterial colonies are placed in urea broth. A positive result is indicated by a change in color within 30 minutes.[27]

H_2S Test- Bacteria with sulfur-reducing compounds exhibit the property of hydrogen sulfide (H_2S) production. The detection of hydrogen sulfide production involved inserting a single colony from each isolate into Triple Sugar Iron Agar (TSI) slants (HiMedia, M0211), prepared under the manufacturer's instructions. The slants were then incubated at 37 °C for 24 hours in duplicate, with a non-inoculated tube serving as the negative control. A positive reaction was evidenced by the emergence of a black coloration.[26]

Citrate (CAU) Test- This test is valuable for identifying microorganisms with the capacity to utilize citrate as an energy source. Citrate agar is employed, which includes citrate and inorganic ammonium as carbon and nitrogen sources, respectively. The CAU test assists in identifying microorganisms that produce the enzyme citrate permease, converting citrate into pyruvate, subsequently entering the organism's metabolic cycle, leading to energy production and visible growth on the culture medium. As the microorganism utilizes citrate, the conversion of ammonium salts generates NH_3, causing an increase in the medium's pH. This pH change is reflected in the colour shift of bromothymol blue from green to blue when the pH exceeds 7.6. Bromothymol blue serves as an indicator in the CAU test.[28]

Methyl Red Test- Certain bacteria utilize glucose and transform it into various types of acids, including lactic acid (LA), acetic acid (AA), and formic acid (FA) as the final products. Initially, glucose is converted to pyruvic acid, and the subsequent acid produced depends on the bacterial species. The acid production leads to a decrease in the medium's pH, causing a color change in methyl red from yellow to red. This alteration signifies the bacteria's capability to utilize the glucose present in the culture medium.[29]

Indole test- The experiment aims to showcase the proficiency of specific bacteria containing the enzyme tryptophanase, which can effectively break down and deaminate tryptophan, resulting in the generation of indole, pyruvic acid, and ammonia. Indole, characterized as a benzyl pyrrole, stands out as one of the end products derived from the metabolic degradation of the amino acid tryptophan. By adding Kovac’s reagent, the emergence of a vivid fuchsia-

pink ring at the boundary between the reagent and the broth within moments of introducing the reagent signifies the existence of indole and serves as a positive indication in the test.[30]

Sugar fermentation test (dextrose, maltose, sucrose, D- mannitol)- A test is conducted to assess an organism's capacity to ferment a particular carbohydrate included in a basic medium, leading to the production of acid with or without the presence of visible gas. A positive outcome is characterized by a color shift to pink, accompanied by or without the formation of gas in Durham's tube. Conversely, a negative result is denoted by the presence of growth but without any alteration in colour.[30]

Plant extract

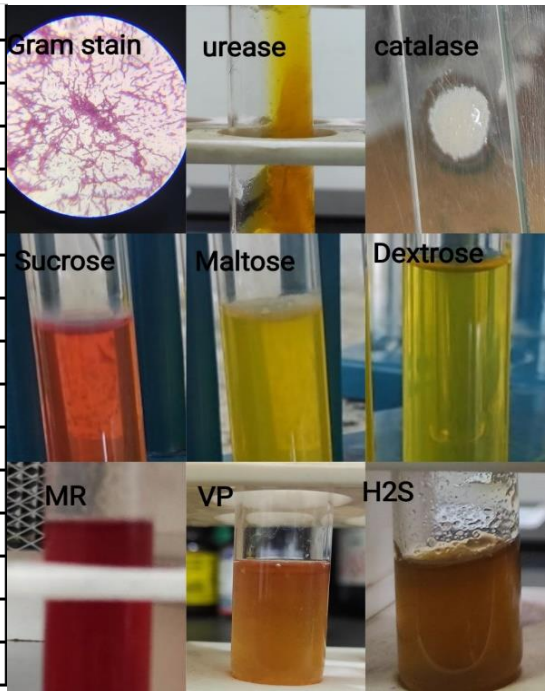
Six plant samples (Cardamom, cloves, ginger, onion, turmeric, tulsi/holy basil) were taken according to our interest, previous knowledge and availability, most of them contain essential oils as the main active ingredient. For extraction, there are some main techniques- The conventional extraction technique (Hydrodistillation and Soxhlet extraction), the Advanced extraction technique (Enzyme assisted extraction, Supercritical extraction, solar energy-based extraction and Ultrasound-assisted extraction), and the Maceration Extraction Technique.[19] We used the Maceration extraction technique here. A sample, consisting of a blend of plant-derived phytochemicals, was acquired through the extraction of specific plant parts. The specimens underwent a drying process in an incubator set at 37°C for 24 hours, followed by grinding into a fine powder. Subsequently, the plant material was dissolved in water (1:10, W/V), and the resulting mixtures were securely stored in sterilized closed conical flasks at room temperature for 2 days to prevent evaporation. After 2 days, the mixtures underwent filtration using Whatman no.1 filter paper and were kept in the refrigerator for further uses.[31]

RESULTS

Salmonella was identified by biochemical tests- a negative Gram stain, positive motility, flagella were present, a positive catalase test, positive MR and sugar fermentation that were dextrose, maltose and D- mannitol all positive. the results are listed below in Table No. 2-

Table 2: & Fig No. 1 - Biochemical Tests of Bacteria (Salmonella).

S. No.	Biochemical Test	Positive	Negative
1	Gram stain	×	✓
2	Motility	✓	×
3	Flagella	✓	×
4	Catalase	✓	×
5	Urease	×	✓
6	Citrate	×	✓
7	MR	✓	×
8	VP	×	✓
9	Indole	×	✓
10	Sucrose	×	✓
11	Dextrose	✓	×
12	Lactose	×	✓
13	Maltose	✓	×
14	D-Manitol	✓	×
15	H2S	×	✓



The increasing apprehension regarding health and disease has spurred the emergence of natural antimicrobials aimed at managing both foodborne pathogens and spoilage microorganisms. Cloves, Turmeric, Cardamom, Ginger, Onion and Tulsi are used in Ayurveda and other Herbalism. They all show good inhibitory activity in bacteria.

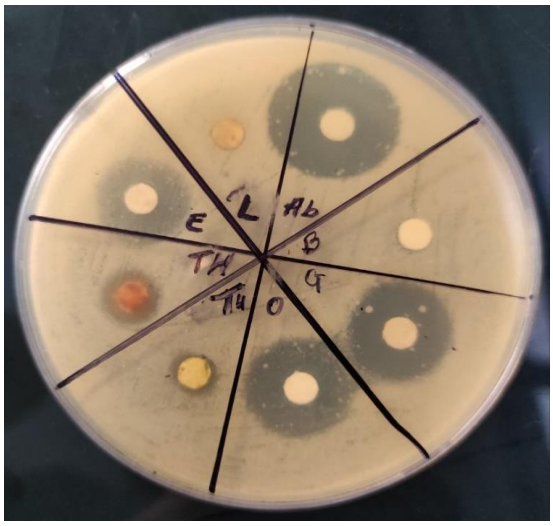


Fig. No. 2: AST Plate.

The antibacterial Susceptibility Test was done twice and the results were slightly different both times. The mean value and Standard Deviation of both experiments are shown in Table No. 2.

Table 2: Mean Value and Standard Deviation of AST.

S. No.	Samples	Zone of inhibition Mean Value (mm)	Standard Deviation
1	Blank (B)	0	0
2	Antibiotic Ab)	23	1
3	Cloves (L)	0	0
4	Cardamom (E)	17	1
5	Turmeric (Th)	9	1
6	Tulsi (Tu)	7	0
7	Onion (O)	17.5	0.5
8	Ginger (G)	17.75	0.75

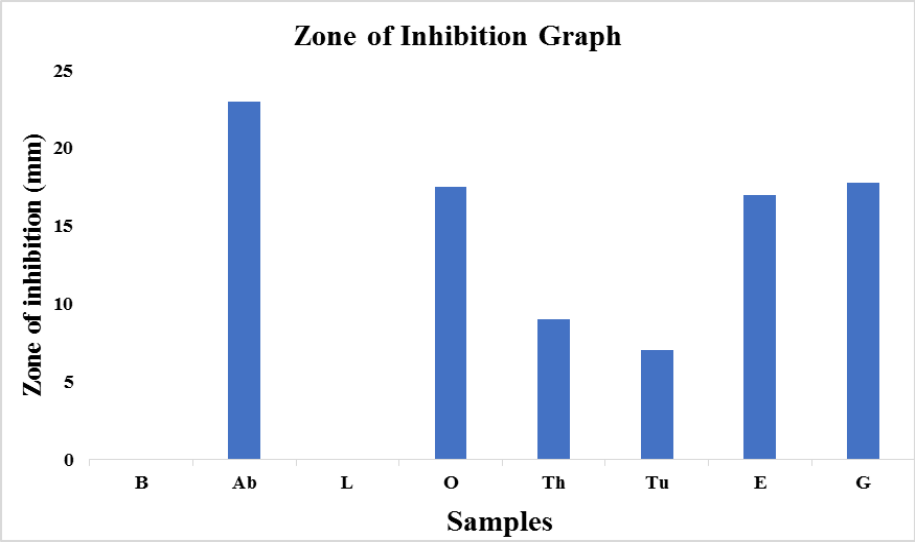


Fig. 3: Zone of Inhibition Graph of AST.

In summary, salmonella is a pathogenic bacteria that causes typhoidal and non-typhoidal diseases. To decrease the efficiency of bacteria some medicinal plants can be used which are way more effective for Salmonella. In this study, we treated salmonella with aromatic plants-Ginger, Onion, Cardamom, Turmeric, Tulsi and clove. An antibiotic (amoxicillin) was taken 30µg for the reference which is very effective for Salmonella. Aromatic plants degraded the zone of salmonella Ginger (17.75mm), Onion (17.5mm), Cardamom (17mm), Turmeric (9mm), Tulsi (7mm) and clove (0mm) respectively. results show that ginger, onion, and cardamom have high antibacterial properties and others have low compared to these.

DISCUSSIONS

Medicinal plants continue to hold significant significance in the everyday existence of individuals residing in developing regions of Asia and Africa. These plants serve not only as supplements or alternatives to modern medical interventions, which are frequently lacking, but also contribute to the well-being and safety of local communities. Consequently, these

plants play essential roles in daily routines and are intricately linked to various social, cultural, and economic aspects associated with existence, aging, ailments, and mortality. The utilization of medicinal plants extends to the treatment and identification of diseases and infections.[32] Throughout history, plants have consistently served as abundant reservoirs of reliable and secure medicinal remedies.[33] The distinct antibacterial activities found in essential oils from various plants can be attributed to the impact of carvacrol and thymol.[34] The inclusion of cardamom in food should be promoted due to its polyphenol profile which may help in improving the antimicrobial status, shelf life, and quality of products as well as alleviating oxidative stress and various lifestyle-related disorders.[19] Ginger and its polyphenols have demonstrated the ability to impact various signaling molecules, forming a foundation for their application in addressing complex human diseases with multiple contributing factors. Moreover, most of the known activities of ginger components are based only on in vitro and in vivo studies, except for a few clinical studies in human subjects.[21] Turmeric, derived from the rhizome of the medicinal plant Curcuma longa indigenous to India, has long been recognized in Ayurveda and Unani for its multifaceted health benefits. Renowned for its anti-inflammatory, carminative, hepatoprotective, anticancer, antioxidant, and antilipidemic properties, turmeric has been a staple in traditional medicine. Within its rhizome extract, a group of phenolic diarylheptanoids, collectively known as curcumoids, is present. The primary active constituent among these compounds is curcumin, or diferuloylmethane. Recent scientific investigations have reaffirmed the medicinal significance of turmeric, highlighting its antibacterial properties.[36]

CONCLUSION

Pathogenic bacteria are increasing nowadays day by day. Salmonella is one of these pathogenic bacteria which causes typhoidal and non-typhoidal infections. When transmitted to humans, pathogens can induce diseases, and their treatment becomes challenging owing to the emergence of antibiotic resistance. Cloves, Turmeric, Cardamom, Ginger, Onion and Tulsi are aromatic plants, antimicrobial activity found in these plants includes Eugenol, Flavonoids, Phenolic acids, Antioxidants, Terpenes, Carvacrol, Limonin etc. Antimicrobial activity found in these plants are high so The growth of selected bacteria (Salmonella) was inhibited by these plant extracts. The antibacterial activity of these plants can be correlated with the results of AST plates.

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