



Study of antimicrobial activity of some medicinal plants against *Escherichia coli*, *Klebsiella* sp., *Staphylococcus* sp. and *Pseudomonas* sp.

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Abstract

In recent years, renewed interest has been shown in the use of medicinal plants and scientific studies are being designed to explain some of the curative phenomena associated with traditional herbal remedies. Most drugs utilized by people all over the world are of plant origin. The use of herbal remedies has increased significantly in the last one decade. This has led to the production of herbal products. The microbial activity of curing all manners of microbial diseases were assessed. The herbal remedies were discovered to eliminate the contamination done by the following microorganisms: *Escherichia coli*, *Klebsiella* sp., *Staphylococcus* sp. and *Pseudomonas* sp. The distilled water, ethanol, and aqueous extract of three medicinal plants were evaluated for activity against medically important bacteria such as *Escherichia coli*, *Klebsiella* sp., *Staphylococcus* sp. and *Pseudomonas* sp. The *in vitro* antimicrobial activity was performed by agar well diffusion method and disc diffusion method. The plates are incubated at 37°C in incubator. The zones of inhibition were observed after this and observe the susceptibility towards the herbal drugs. The ethanolic extract of garlic inhibited the growth of both gram positive and gram-negative test bacterial cultures. The maximum activity was noted against *Staphylococcus*, *Escherichia coli* and minimum antibacterial activity against *Pseudomonas*. A zone of 12mm was recorded *Escherichia coli*, *Staphylococcus* by ethanol.

Keywords: Anti-microbial activity, well- diffusion method, disc- diffusion method, zone of inhibition, *Escherichia coli*, *Staphylococcus* sp., *Pseudomonas* sp., *Klebsiella* sp

Introduction

Before there was modern-day medicine and its pharmacopeia of synthetic drugs, there were plants, and ancient civilizations knew how to use them strategically to treat common ailments and even life-threatening diseases. Later, during the 1800s and early 1900s, the knowledge of herbal medicine was passed down from one generation to the next. Typically, the woman of the house was well versed in the use of herbs for healing, and would act as the family's physician not only to treat illnesses but also to prepare various herbal wellness tonics and other remedies. Today, the World Health Organization (WHO) estimates that 80 percent of the world's population still uses

traditional remedies, including plants, as their primary health care tools. Meanwhile, the majority of new drugs (70 percent) introduced in the US are derived from natural products, primarily plants.

Approximately 250,000 species of flowering plants and only 5000 have had their pharmaceutical

potential assessed. The treatment of infectious diseases with antimicrobial agents continues to present problems in modern- day medicines with many studies showing a significant increase in the incidence of bacterial resistance to several antibiotics. (C.M. Kunin, 1993)^[3]

Lists of medicinal plants used to evaluate antimicrobial activity:

Sl. No.	Local Name	English Name	Botanical Name	Parts of plant used
1.	Rosun	Garlic	<i>Allium sativum</i>	Bulb
2.	Peyaj	Onion	<i>Allium cepa</i>	Bulb
3.	Nim	Neem	<i>Azadirachta indica</i>	Leaf

Azadirachta indica (Neem)

Azadirachta indica is a tree in the mahogany family Meliaceae. Neem is the most useful traditional medicine as a source of many therapeutic agents in the Indian culture and grows well in the tropical and semi-tropical countries. Its twigs are used as tooth brush and are widely used in the Indian sub-continent. Earlier studies on neem have showed that it contains active substances in almost every part of the seeds, leaves, roots, bark, trunk and branches with multiple

medicinal properties. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products. *A. indica* is used for the treatment of diabetes like neem biscuits and shows the potential role of anti-diabetic activity. Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise. (Mishra A., *et al*, 2013)^[1].



Azadirachta indica (Neem)

Allium cepa (Onion)

Since ancient times onion (*Allium cepa*, L.) have been an important dietary resource and have also been of interest for medical purposes. *Allium* is the largest and important representative genus of the Liliaceae family comprises 450 species. Onion (*Allium cepa*) is a bulbous plant widely cultivated in almost every country of the world. Onions are easily propagated, transported and stored. Onions are effective against common cold, heart disease, diabetes, osteoporosis, coughs and sore throat. It is rich in proteins, carbohydrates, sodium, potassium and phosphorus. Onion was consumed throughout Europe during the middle ages and was later thought to guard against evil spirits and the plague, probably because of their strong odor. Folk healers traditionally used onion to prevent infections is among the oldest cultivated plants used both as a food and for medicinal applications. The plant is used as traditional remedy in the treatment of various disorders so it has particular medicinal importance. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties. Onion bulbs contain a good number of phytochemicals, most of which are hydrocarbons and their derivatives. Several authors have reported pharmaceutical activity of extracts of *Allium cepa* including Anti-tumor, anti-diabetic, antioxidant, antimicrobial, anti-allergic and molluscicidal activity. *In vitro* studies have shown onion to possess antibacterial, antiparasitic, and antifungal activity. (Eltaweel Mohamed, 2013)^[2].



Allium cepa (Onion)

Allium sativum (Garlic)

Garlic is an earth wonder the name of which has been lived by Turks without forgetting during long history and extensive geography of them (Akciçek, 2006)^[4]. Garlic (*Allium sativum*) the land of which is said as Middle and West Asia steps has a place among eldest crop plants. This plant, which is of great medical importance takes place inside many foods especially, meat ones due to its sharp odour, appetizer property and bitter taste and gives flavor to them. Garlic, its calorie value is 140, has 63.8 g water, 28.2 g carbohydrate, 5.3 g protein, 0.2 g oil and 11 g cellulose in its 100 g (Baytop, 1999; Kutevin and Turks, 1987)^[5].

Garlic is plants, which kills bacteria, fungus, parasites and lowers glycaemia and cholesterol and have liver protector property and includes antitumor agents. Garlic, with >200 chemical substance in its body, has the capacity of protecting human body against many illnesses. It's declared that garlic, as an anti-bacterial agent, is effective against many more grams negative and gram-positive bacteria like *Helicobacter pylori*, *E. coli*, *Lactobacillus casei*, etc and that this effect is sourced from allicin inside it. (Cellini *et al.*, 1996; Lermar *et al.*, 2005)^[7, 8].



Allium sativum (Garlic)

In developed countries, good hygienic practices, adequate preservative technique for processed foods and use of antimicrobial substances such as antibiotics are the various ways of control and treatment. Traditional medicine is practiced and plants have been exploited for the treatment of many infections and diseases. Plants readily synthesize substances for defense against attack by insects, herbivores microorganisms (Majorie, 1999)^[10]. The antimicrobial nature of these substances has been well documented. (Aboaba *et al.*, 2006)^[9].

The aim of this research work was to find out possible antimicrobial action of the extracts of some edible plants traditionally used as herbal medicine on the growth of various microorganisms.

Methodology

- **Collection of bacterial isolates:** The bacterial isolates such as *Klebsiella*, *E. coli*, and *Pseudomonas* sp. were collected from different sites. 10 isolates were collected during this work.
- **Identification of bacterial species:** A series of morphological, gram staining and bio-chemical tests such as Simmon Citrate Agar, Gelatin Powder, Triple

Sugar Iron Agar, Skim Milk, Urea Agar Base, Coagulase Mannitol Salt Agar were performed to identify the suspected isolates.

- **Preparation of plant extracts of neem:** The fresh parts of plants such as leaves were collected and washed several times with distilled water. Leaves were cut and paste was made by using mortar- pestle. The stored leaf extracts were impregnated on filter paper and air- dried under laminar for overnight. These filter paper discs were used as herbal discs.
- **Preparation of Aqueous and Ethanolic Extracts:** Two spices such as garlic (*Allium sativum*), and Onion (*Allium cepa*) were washed first by distilled water and then by 95% ethanol. Both the spices were crushed and sieved through mesh cloth to get the filtrate. The filtrate was heated at 40-50°C using water bath, until the thick paste is formed which is considered as 100% concentration of extracts. Extracts of both the spices were stored in 4° C in refrigerator. Extracts of these spices were further diluted to make different concentrations such as 100%, 75%, 50% and 25% by mixing distilled water. The ethanolic extract was prepared following same procedure with the exception of solvent which was 95% ethanolic instead of sterilized distilled water.
- **Isolation and identification of bacteria:** Dilution series of sewage samples were prepared in test tube as 10^{-1} to 10^{-4} from which only 10^{-2} and 10^{-4} were inoculated by Spread Plate Method. Each dilution was inoculated in 4 different media to differentiate gram

positive and gram-negative bacteria from clinical and non- clinical specimens and Nutrient Agar Media which is a general-purpose medium to observe the colony morphology. In order to obtain pure cultures with homogenous colonies of bacteria, they were cultured in their respective and specialized media such as *E. coli* in EMB, *Staphylococcus* in Mannitol Salt Agar, *Pseudomonas* in Selective *Pseudomonas* Agar and *Klebsiella* in Selective *Klebsiella* Agar.

- **Herbal Drug Sensitivity Test Using Disc Diffusion Method:** Different prepared Antibiotics discs from different plant extracts were taken and placed in Muller Hinton Agar plates to determine the inhibition zone. Different concentrations such as 25%, 50%, 75% and 100% were placed in the corners of the plate and incubated at 37° C for 24 hours.
- **Assay of antimicrobial activity using Agar Well Diffusion Method:** The sterilized Muller Hinton Agar was poured into sterile petriplates; human pathogens were swabbed on the respective plates. The wells were punched over the agar plates using sterile gel puncher at several of each plant extracts were added to the wells. The plates were then incubated at 37° C for 24 hours and then the diameter of inhibitory zones was measured around the well and recorded.
- **Observations**

Percentage of extract using in antibiotics

Sl. No.	Plant Name	Extract Concentration (Distilled water and ethanolic)			
1.	<i>Allium sativum</i>	25%	50%	75%	100%
2.	<i>Azadiachta indica</i>	25%	50%	75%	100%
3.	<i>Allium cepa</i>	25%	50%	75%	100%

Minimum Inhibition Zone of extract against given concentration

Table 1: Garlic extract in ethanol by Disc Diffusion Method

Sl. No.	Garlic extract in Ethanol	Concentration and zone of inhibition			
	Organisms	25%	50%	75%	100%
1.	<i>E. coli</i>	11 mm	9 mm	12 mm	12 mm
2.	<i>Klebsiella</i>	6 mm	7 mm	9 mm	10 mm
3.	<i>Staphylococcus</i>	13 mm	12 mm	15 mm	13 mm
4.	<i>Pseudomonas</i>	R	R	R	R

Table 2: Onion extract in ethanol by Disc Diffusion Method

Sl. No.	Onion extract in Ethanol	Concentration and zone of inhibition			
	Organisms				

		25%	50%	75%	100%
1.	<i>E. coli</i>	R	R	R	R
2.	<i>Klebsiella</i>	R	R	R	R
3.	<i>Staphylococcus</i>	.05 mm	.07 mm	.08 mm	1.1 mm
4.	<i>Pseudomonas</i>	R	R	R	R

Table 3: Onion extract in ethanol by Disc Diffusion Method

Sl. No.	Onion extract in Ethanol	Concentration and zone of inhibition			
	Organisms	25%	50%	75%	100%
1.	<i>E. coli</i>	.06 mm	.06 mm	.07 mm	.05 mm
2.	<i>Klebsiella</i>	R	R	R	R
3.	<i>Staphylococcus</i>	.02 mm	.02 mm	.02 mm	.02 mm
4.	<i>Pseudomonas</i>	R	R	R	R

Table 4: Garlic extract in distilled water by Disc Diffusion Method

Sl. No.	Garlic extract in distilled water	Concentration and zone of inhibition			
	Organisms	25%	50%	75%	100%
1.	<i>E. coli</i>	.04 mm	.03 mm	.01 mm	.01 mm
2.	<i>Klebsiella</i>	.02 mm	.01 mm	.04 mm	.03 mm
3.	<i>Staphylococcus</i>	.07 mm	.09 mm	1 mm	1.5 mm
4.	<i>Pseudomonas</i>	.02 mm	.03 mm	.04 mm	.05 mm

Table 5: Onion extract in distilled water by Disc Diffusion Method

Sl. No.	Garlic extract in distilled water	Concentration and zone of inhibition			
	Organisms	25%	50%	75%	100%
1.	<i>E. coli</i>	R	R	R	R
2.	<i>Klebsiella</i>	R	R	R	R
3.	<i>Staphylococcus</i>	.01 mm	.01 mm	.02 mm	.03 mm
4.	<i>Pseudomonas</i>	R	R	R	R

Table 6: Neem extract in distilled water by Disc Diffusion Method

Sl. No.	Garlic extract in distilled water	Concentration and zone of inhibition			
	Organisms	25%	50%	75%	100%
1.	<i>E. coli</i>	R	R	R	R
2.	<i>Klebsiella</i>	R	R	R	R
3.	<i>Staphylococcus</i>	R	R	R	R
4.	<i>Pseudomonas</i>	R	R	R	R

Antimicrobial activity using Agar Well Diffusion Method

Table 7: Onion extract in ethanol by Well Diffusion Method

Sl. No.	Onion extract in ethanol	Zone of inhibition in mm.
	Organisms	
		100% concentration
1.	<i>E. coli</i>	14 mm
2.	<i>Klebsiella</i>	17 mm
3.	<i>Staphylococcus</i>	15 mm
4.	<i>Pseudomonas</i>	9 mm

Table 8: Neem extract in ethanol by Well Diffusion Method

Sl. No.	Neem extract in ethanol	Zone of inhibition in mm.
	Organisms	
		100% concentration
1.	<i>E. coli</i>	14 mm
2.	<i>Klebsiella</i>	18 mm
3.	<i>Staphylococcus</i>	R
4.	<i>Pseudomonas</i>	10 mm

Table 9: Garlic extract in distilled water by Well Diffusion Method

Sl. No.	Garlic extract in distilled water	Zone of inhibition in mm.
	Organisms	
		100% concentration
1.	<i>E. coli</i>	10 mm
2.	<i>Klebsiella</i>	11 mm
3.	<i>Staphylococcus</i>	21 mm
4.	<i>Pseudomonas</i>	R

Table 10: Onion extract in distilled water by Well Diffusion Method

Sl. No.	Onion extract in distilled water	Zone of inhibition in mm.
	Organisms	
		100% concentration
1.	<i>E. coli</i>	R
2.	<i>Klebsiella</i>	16 mm
3.	<i>Staphylococcus</i>	R
4.	<i>Pseudomonas</i>	R

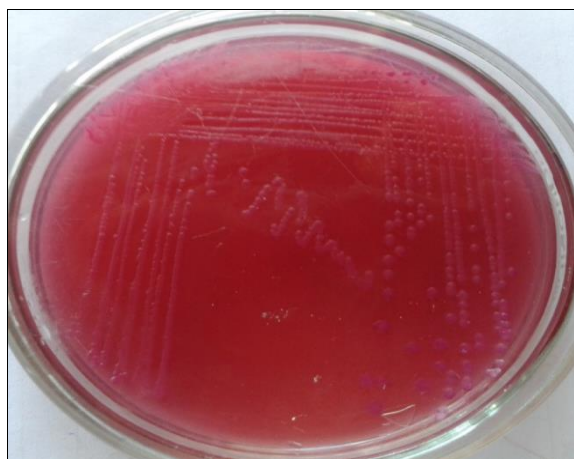
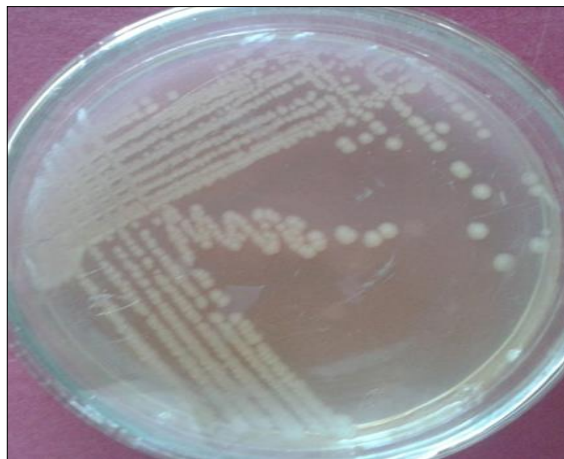
Table 11: Neem extract in distilled water by Well Diffusion Method

Sl. No.	Onion extract in distilled water	Zone of inhibition in mm.
	Organisms	
		100% concentration
1.	<i>E. coli</i>	R
2.	<i>Klebsiella</i>	14 mm
3.	<i>Staphylococcus</i>	R
4.	<i>Pseudomonas</i>	R

Table 12: Garlic extract in Ethanol by Well Diffusion Method

Sl. No.	Onion extract in Ethanol	Zone of inhibition in mm.
	Organisms	
		100% concentration
1.	<i>E. coli</i>	12 mm
2.	<i>Klebsiella</i>	14 mm
3.	<i>Staphylococcus</i>	23 mm
4.	<i>Pseudomonas</i>	R

Photographs

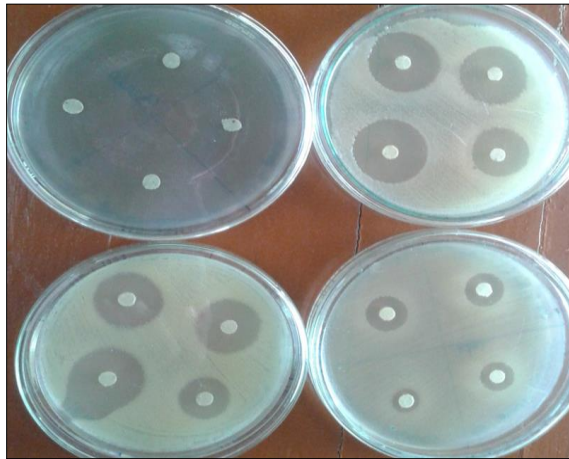
Sub culturing of *E. coli* in MacConky AgarSub culturing of *E. coli* in Nutrient Agar



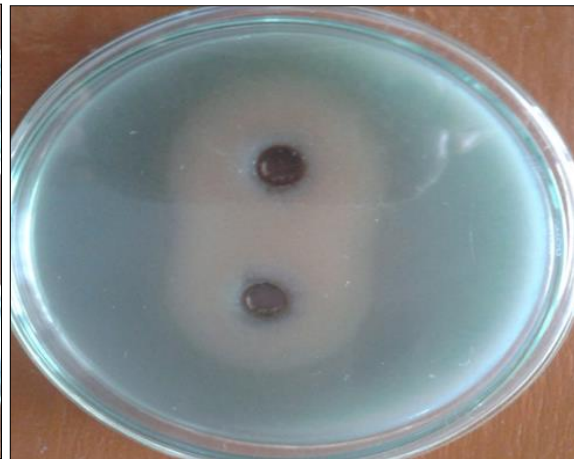
Biochemical test of various micro-organisms



Plate showing resistance towards garlic herbal plant in ethanol extract



Herbal antibiotic sensitivity against microorganisms



Well method of herbal extracts

Result and Discussion

The study was performed to investigate the antibacterial properties of some medicinal plant extracts to *Klebsiella*, *E. coli*, *Staphylococcus*, *Pseudomonas* sp. isolates. A total of 6 herbal extracts prepared from 3 plants were used in this study to screen their antibacterial activity to the various bacterial sp. The activity of ethanol and aqueous extracts were investigated using Disc Diffusion method and Agar Well Diffusion Method.

The ethanolic extract of garlic inhibited the growth of both gram positive and gram-negative test bacterial cultures. The maximum activity was noted against *Staphylococcus*, *E. coli* and minimum against *Pseudomonas*. A zone of 12 mm was recorded *E. coli* and *Staphylococcus* by ethanol

The collected different fractions of garlic methanol extract were subjected to antibacterial assay by well diffusion using MHA plates. About 8 ul of the fractions were loaded in plates. The inhibitory activity was observed by the zone developed on the plates. *E. coli* was found to exert an inhibitory effect of 12 mm and *Staphylococcus* with a zone of 11 mm. Overall increase of zone of inhibition was noticed for increased concentrations for *E. coli* and *Staphylococcus* respectively.

It is evident from our study that onion has a less antibacterial activity than garlic and neem. At 100% and 50% concentrations of onion, the results showed better growth against *Staphylococcus*, while when we use 25% concentration of onion the results showed growth of microbial strains. The isolates were subjected in to antibiotic

susceptibility test to identify the resistant and sensitive pattern of organisms. From the result, we conclude the garlic ethanolic extract were found to be more sensitive for *E. coli*.

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