

Study of Antimicrobial effect of some leafy vegetables-a comparative study

Pranjali Rangari¹ and Sneha Khadse^{2*}

¹Kamla Nehru Mahavidyalaya, Nagpur, MS, India

²Project Assistant at CSIR – NEERI, Nagpur, MS, India

*Email id: khadsesneha43@gmail.com

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ABSTRACT

The present study deals with the antimicrobial effect of some leafy vegetables that is a comparative study of leafy vegetables. The plant samples were collected from local vegetables vendors. They were categorized as Seasonal (Monsoon) plants- *Amaranth leaves (chawlai)*, *spinach*, *fenugreek leaves (methi)*, *daikon leaves (muli)*. The method of agar well diffusion method was used for checking the antimicrobial activities of test samples. Each test organism was grown by inoculating the bacterial culture into fresh sterile Nutrient media and incubating overnight at 37 degree centigrade. These cultured organisms were then used for the study. The effective and determinative anti-microbial activity against the selected bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*) was extracted with aqueous and acetone. The acetone extract was found more active against all the test bacterial strains than hot water extract.

Keywords: Spinach (*Spinacia oleracea*), Methi (Fenugreek Leaves), Daikon (*Raphanus sativus*), Chawali (Amaranth Leaves)

INTRODUCTION

Many leafy vegetables from part of people's diet, some of which are available throughout the year and others more prevalent during certain seasons of the year, especially the monsoon vegetables seasons are declining due to a belief that they are 'krumikarak' which means that they enhance the intestinal parasites. Some of them have also been observed to have antimicrobial activity. Since antibiotic resistance has long been a significant problem in treatment of bacterial infection, there is always a need for newer and safer options, having easy administration and lesser side effects.

Green leafy vegetables have been used as a medicine since ancient times and have been playing a very important role in our diet and nutrition. They are the most readily available sources of carbohydrates, fats, important

proteins, vitamins, minerals, essential amino acids, and fibers. Their bioactive substances have a wide range of biological functions, including antioxidant and antimicrobial activities and can be helpful in management of oxidative stress and age related human ailments. They are a rich source of carotene, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron and phosphorus. Being a photosynthetic tissue, leafy vegetables have a higher level of vitamins K when compared with other fruits and vegetables due to direct involvement of vitamin K (phyloquinone) in photosynthesis. Vegetables as medicinal plants contain none or less toxic effects, and have the ability to synthesize several secondary metabolites of relatively complex structure possessing antimicrobial activities. Green leafy vegetables are also rich in compounds having anti-diabetic, antihistamine, anti-carcinogenic and hypolipidemic properties and possess preventive and curative properties against cardiovascular disease, aging, obesity, hypertension, insomnia and ageing. Leafy vegetables are a natural source of antioxidants and rich in phytochemicals. Also in recent years, multiple drug resistance in human pathogenic microorganism has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of various diseases. The present work has therefore been designed to investigate the antibacterial effect of four leafy vegetables.

Four different vegetables

1) Spinach (*Spinacia oleracea*), 2) Methi (Fenugreek Leaves) 3) Daikon (*Raphanus sativus*), 4) Chawali (Amaranth Leaves)

MATERIAL AND METHOD

1) Collection and Preparation of plant extract: - The plant samples were collected from local vegetable vendors. They were categorized as Seasonal (Monsoon) plants- *Amaranth leaves (chawlai)*, *spinach*, *fenugreek leaves (methi)*, and *daikon leaves (muli)*.

Their edible & non-edible parts were separated and only the edible parts were selected for further treatment. The edible parts such as leaves were kept for drying in a hot air oven at 50°C, over a period of 48 hours to obtain a constant dry weight, after which

they were grinded in a mortar & pestle to a fine powder and were preserved in an amber colored bottle under refrigeration. The plant samples (1gm) were soaked in 10ml of sterile warm distilled water, dissolved plant extract properly and then it can be filtered by cotton cloth or muslin cloth.

2) Acetone Extraction of the plant extracts: The plant samples (1g) were soaked in solvent acetone 20ml and were kept for incubation overnight under dark room condition. The solutions were filtered through Whatmans filter paper, Grade 1 and the extracts were preserved in amber colored bottles till further use.

3) Antimicrobial study: The bacterial species used for the test were *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E.coli*) & *Klebsiella pneumoniae* (*K.pneumoniae*) and *pseudomonas aeruginosa*. The microorganisms were grown overnight at 37° C, in sterile Nutrient Broth at pH 7.4.

4) Culture media and inoculums preparation: Nutrient Broth and Nutrient Agar were prepared as per the standard procedure. A modified method of agar well diffusion method was used for checking the antimicrobial activities of test samples. Each test organism was grown by inoculating the bacterial culture into fresh sterile media and incubating overnight at 37°C. These cultured organisms were then used for the study.

5) Testing for antibacterial activity: The Antimicrobial susceptibility testing was done by using the Agar well diffusion method to detect the presence of antibacterial or antifungal activities of the samples (R.C.Jagessar et al., 2008). The sterile Nutrient Agar media is poured into the Petri plates. As soon as the agar was solidified a well was prepared into the plates with the help of a borer that was sterilized with alcohol and flame. A sterile swab was used to evenly distribute bacterial or fungal culture over the surface of the sterile nutrient agar plates. The plates were allowed to dry for 15 minutes before use in the test. The test samples were introduced into the wells. The antibiotics were introduced into the centre of the well for control. The plates were labelled and incubated overnight at 37 °C. Microbial growth was determined by measuring the diameter of the zone of inhibition. The diameter of inhibition zones was measured in mm and the results were recorded.

RESULT AND DISCUSSION:

In particular the antimicrobial action of each compound was different in intensity and specificity. The extract from *Spinach*, *fenugreek*, *daikon*, *amaranth* shows the effective and determinative

anti-microbial activity against the selected bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*) was extracted with aqueous and acetone. The acetone extract was found more active against all the test bacterial strains than hot water extract.

Table 1: Zone of inhibition from acetone extracts in mm

Test organism used	Spinach	Fenugreek leaves	Daikon leaves	Amaranth leaves	Control
<i>Escherichiacoli</i>	10	18	13	16	3
<i>Staphylococcus aureus</i>	11	11	14	20	10
<i>Pseudomonasaeruginosa</i>	10	12	12	10	4
<i>Klebsiellapneumonia</i>	13	11	0	14	3.5

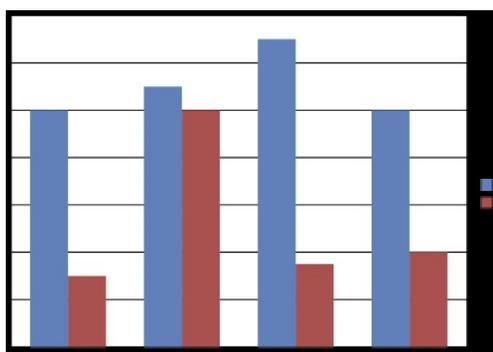


Figure-1: Antimicrobial activity of Spinach

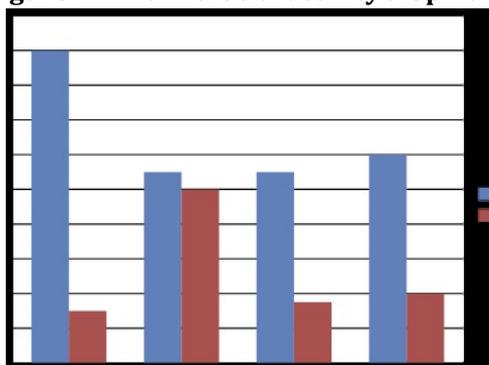


Figure 2: Antimicrobial activity of Fenugreek leaves

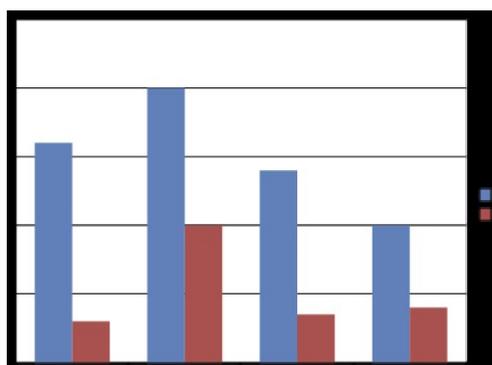


Figure 3: Antimicrobial activity of Daikon leaves

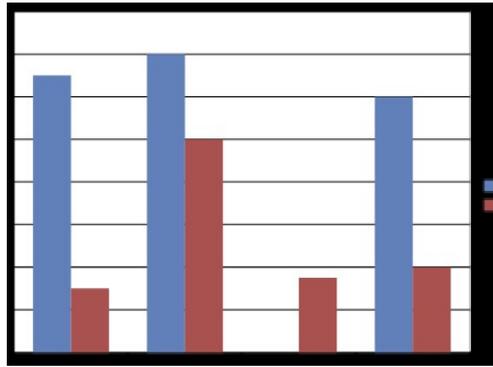
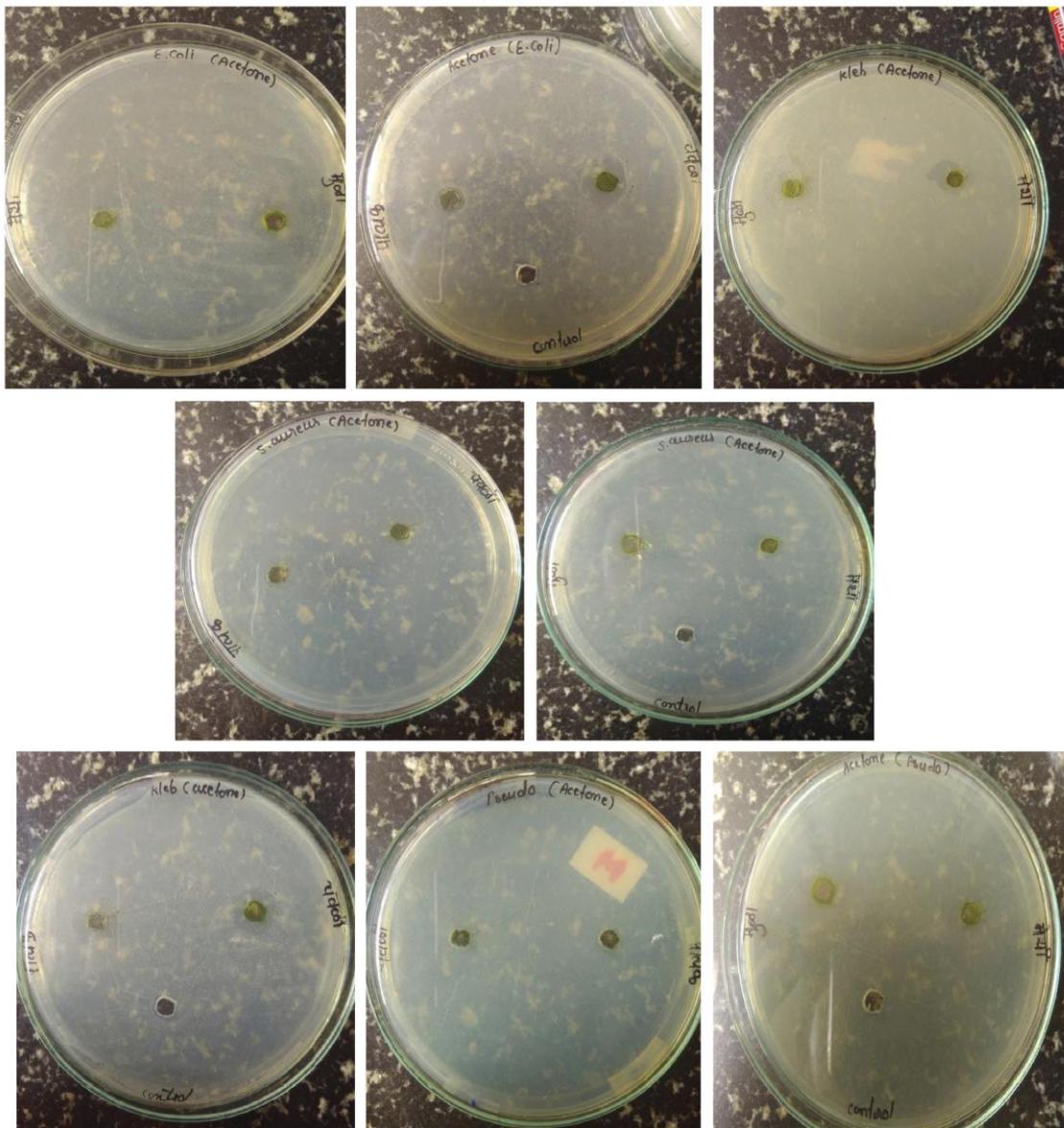


Figure 4: Antimicrobial activity of Amaranth leaves



The result of antibacterial activity for the leafy vegetables, listed in the Table-1. Demonstrated that all the extract of have shown antibacterial activity against the entire set of test organisms used. The

acetone extract of fenugreek leaves (18mm) is more effective against E.coli, than spinach (10mm), daikon leaves(13mm), and amaranth leaves(16mm).The acetone extract of amaranth leaves

(20mm) shows more antibacterial activity against *S.aureus* than rest of the leafy vegetable extract, spinach (11mm), fenugreek leaves (11mm) and daikon leaves (14mm). Also the antimicrobial activity of fenugreek leaves (12mm) and daikon leaves (12mm) is more effective against *pseudomonas aeruginosa*, than other leafy vegetable extract spinach (10mm) and amaranth leaves (10mm). The acetone extract of amaranth leaves (14mm) having more antibacterial activity against *klebsiella pneumoniae* than other leafy vegetables such as spinach (13mm) and fenugreek (11mm), but the daikon leaves does not having any antimicrobial activity against the *klebsiella*, so zone of inhibition is not shown.

CONCLUSION

The solvent extract that is acetone have shown measurable antimicrobial activity against all the organism strains, than hot water extract, so the zone of inhibition were observed and it concluded that, From the observation, the acetone extract fenugreek leaves (18mm) was found to be best against *E.coli*, than other leafy vegetables i.e. spinach, daikon leaves and amaranth leaves. The acetone extraction of amaranth leaves (20mm) was found to be best against *S.aureus* than other leafy vegetables i.e. spinach, fenugreek leaves and daikon leaves. Also the acetone extract of fenugreek leaves (12mm) and daikon (12mm) was found to be more effective against *pseudomonas aeruginosa* than other leafy vegetables such as spinach, amaranth leaves. The acetone extract of amaranth leaves was found to be best against *klebsiella* than other leafy vegetables that are spinach and fenugreek with exception of daikon leaves which did not show any inhibition against *klebsiella*. So comparatively, amaranth leaves and fenugreek leaves extraction in acetone shows more zones of inhibition and gives best anti-microbial activity against all the organism stains used. But spinach and daikon leaves also have an effective antibacterial activity.

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