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# Anti-Microbial Effects of Some Leafy Vegetables - A Comparative Analysis

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## Abstract

*Many plants are known to inhibit multiplication of certain microbes that can cause health hazards in humans. These plants owe their antimicrobial properties mostly to the secondary metabolites such as alkaloids, glycosides, tannins and volatile oil present within. These compounds can be extracted from plants using organic solvents. Monsoon plants, mostly the leafy vegetables, are often credited for their high fiber and mineral content but are less investigated for their efficacy in controlling the growth of pathogenic microbes. The current research aims at identifying some plants that are available only during the monsoon and investigating their ability to act as antimicrobial agents. A paper disc method is employed for the purpose and the plant extracts are made using three organic solvents namely ethanol, acetone and chloroform. It shows promising results against the pathogenic bacterial strains of Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae used in the experiments. This base data can lead to further investigations to separate and purify secondary metabolite fractions from these plant extracts and identify their active ingredients responsible for their antimicrobial activity.*

**Keywords**: Antimicrobial, secondary metabolites, organic solvents, monsoon plants, pathogenic strains, active ingredients.

## Introduction

The importance of medicinal properties of plant stems from their ability to target certain physiological mechanisms of the human body by providing certain vital phytochemicals[1-4]. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds *(Hill, 1952)*. Many of these indigenous medicinal plants are either used as spices or food. Pregnant and nursing mothers also benefit from the medicinal properties of these plants if included in their diet. Many leafy vegetables form part of people's diet, some of which are available throughout the year and others more prevalent during certain season of the year, especially the monsoon vegetables. However, consumption of leafy vegetables during the monsoon season is declining due to a belief that they are *'krumikarak'* which means that they enhance the growth of intestinal parasites[5]. Certain plants species from the genera Amaranthus, Amorphophallus, Clerodendron and many more have been accredited with therapeutic properties according to the literature survey[6-12]. Some of them have also been observed to have antimicrobial activity[13-15]. 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. Thus the research work presented in this paper makes an attempt at exploring the possibility of an antimicrobial activity of some locally available, less known, leafy vegetables.

## Material and Methods

**Collection and Preparation of plant material:** The plant samples were collected from various markets of Mumbai from local vegetable vendors[16-18]. They were categorized as Seasonal (Monsoon) plants- Math (*Amaranthus viridis,* family- *Amaranthaceae*)[19], Shevra (*Amorphophallus commutatus*, family- *Araceae*) and Bharangi (*Clerodendron indicum,* family- *Lamaiceae*) and All Season plants- Ghol (*Portulaca oleracea*, family- *Portulacaceae*)[20] and Mayalu (*Basella alba var. rubra*, family-*Basellaceae*)[19]. Their edible & non-edible parts were separated and only the edible parts were selected for further treatment. The edible parts i.e. leaves were kept for drying in a hot air oven at 50$^{o}$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[21-23].

**Extraction of the plant extracts:** The plant samples (1g) were soaked in three solvents (ethanol, acetone and chloroform) 20ml each and were kept for incubation for overnight under dark room condition. The solutions were filtered through Whatman® quality filter paper, Grade 1 and the extracts were preserved in amber colored bottles till further use[24,25].

**Antimicrobial study:** The bacterial species used for the test were *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli)* & *Klebsiella pneumoniae (K.pneumoniae)*. The microorganisms were grown overnight at 37$^{o}$ C, in sterile Nutrient Broth at pH 7.4 [26 27].

**Culture media and inoculums preparation:** Nutrient Broth and Nutrient Agar were prepared as per the standard procedure[26,27]. A modified method of Disc diffusion technique by Kirby Bauer was used for checking the antimicrobial activities of test samples[28-30]. Each test organism was grown by inoculating the bacterial culture into fresh sterile media and incubating overnight at 37$^0$C. These cultured organisms were then used for the study.

**Testing for antibacterial activity:** The extracts obtained above were screened for their antibacterial activity in comparison with respective solvents as controls, using the standardized agar disc-diffusion method known as the Kirby Bauer method[29,30]. 1 ml of the diluted sample of tests organisms was spread over the N.A. plate using the spread plate technique. Whatman's Filter paper discs of uniform diameter and thickness were saturated with plant extracts prepared using organic solvents and were placed on the agar surface. The plate's area was divided into a quadrant and three of them were impregnated with the paper discs of pre-defined area, saturated with a particular plant extract made in an organic solvent and were labeled as 'experimental quadrants'. The fourth quadrant contained a paper discs saturated with the organic solvent only, and labeled as the 'control quadrant. The plates were wrapped and were kept for incubation 24 hrs at 37$^o$C.. All tests were carried out in triplicates. The zones of inhibition were observed and measured[31-42].

**Statistical analysis of data:** The data obtained were subjected to ANOVA test (MS-Excel) to determine the difference in the antimicrobial activity of the extracts of monsoon plants and All Season plants. The values are expressed in mean ± SEM All the calculations were performed using Microsoft Excel 2007(Data Analysis)[43-46].

## Results and Discussion

**Seasonal (Monsoon) plants:** The results of antibacterial activity for the Seasonal plants, listed in the Table-1, demonstrate that all the extracts have shown antibacterial activity against the entire set of test organisms used. The ethanol extract of Bharangi plant was more effective (2.85 ± 2.73) against *E.coli* where as the chloroform extract of Math plant was more effective (2.71 ± 2.56) against *K.pneumoniae*. On the other hand, ethanol extract of Shevra plant was ineffective against *S.aureus* while the acetone extract of Shevra plant was ineffective against all the three organisms.

**All Season plants:** The results of antibacterial activity for all season plants, listed in the Table-2, clearly show that all the extracts exhibit antibacterial activity against the entire set of test organisms used. The ethanol extract of Mayalu and Ghol plants was more effective (3 ± 2.08 and 2.57 ± 3.25) against S.aureus. The acetone extract of Ghol plant was more effective (2.71 ± 3.49) against K.pneumoniae while the acetone extract of Mayalu plant was more effective (1.71 ± 0.75) against S.aureus. The chloroform extract of Ghol was more effective (5.85 ± 3.67) against K.pneumoniae while the extract of Mayalu was more effective (4 ± 2.23) against S.aureus. The ethanol, acetone and chloroform extract of Takla was ineffective against all the three organisms.

**Table-1**

**Antibacterial activity of Math, Shevra & Bharangi extracts made in different solvents against the test organisms. Values are expressed as Mean±SEM area of inhibition for 7 replicates per plant. E*: Ethanol; A*: Acetone; C*: Chloroform**

| Bacterial strains | Math (Mean ± SEM) | | | Shevra (Mean ± SEM) | | | Bharangi (Mean ± SEM) | | |
|---|---|---|---|---|---|---|---|---|---|
| | E* | A* | C* | E | A | C | E | A | C |
| *E.coli* | 0.57± 1.13 | 1.71± 2.98 | 2± 1.82 | 0.42± 0.53 | 0± 0 | 0.57± 0.97 | 2.85± 2.73 | 2.14± 2.11 | 1.42± 1.13 |
| *S.aureus* | 0.14± 0.37 | 1.28± 2.98 | 0.14± 0.37 | 0± 0 | 0± 0 | 0.28±0.48 | 1.71± 1.49 | 1.14± 1.46 | 1.85± 1.57 |
| *K.pneumoniae* | 0.14± 0.37 | 1.57± 2.93 | 2.71± 2.56 | 0.85± 1.21 | 0± 0 | 0.57±0.78 | 2.42± 1.27 | 1.42± 1.61 | 1.28± 1.25 |

**Table-2**

**Antibacterial activity of Ghol, Shevra & Takla extracts made in different solvents against the test organisms in terms of inhibition for 7 replicates. Values are expressed as Mean±SEM area of inhibition for 7 replicates per plant. E*: Ethanol; A*: Acetone; C*: Chloroform**

| Bacterial strains | Ghol (Mean ± SEM) | | | Mayalu (Mean ± SEM) | | | Takla (Mean ± SEM) | | |
|---|---|---|---|---|---|---|---|---|---|
| | E* | A* | C* | E | A | C | E | A | C |
| *E.coli* | 0.14± 0.37 | 0.28± 0.75 | 0± 0 | 1.85± 0.89 | 1.42± 1.39 | 2.14± 1.34 | 0± 0 | 0± 0 | 0± 0 |
| *S.aureus* | 2.57± 3.25 | 0.14± 0.37 | 0.28± 0.48 | 3± 2.08 | 1.71± 0.75 | 4± 2.23 | 0± 0 | 0± 0 | 0± 0 |
| *K.pneumoniae* | 0± 0 | 2.71± 3.49 | 5.85± 3.67 | 2.57± 1.81 | 1.28± 1.25 | 2.85± 2.19 | 0± 0 | 0± 0 | 0± 0 |

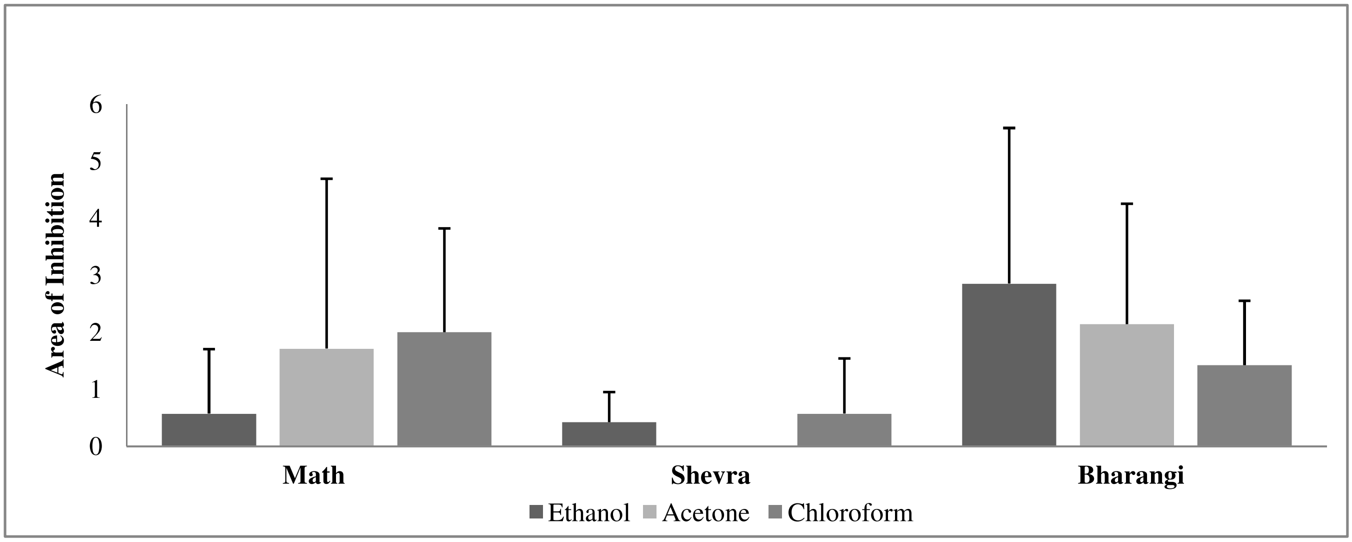


**Figure-1**
**Effect of different solvents extracted from Seasonal vegetables on E.coli growth**

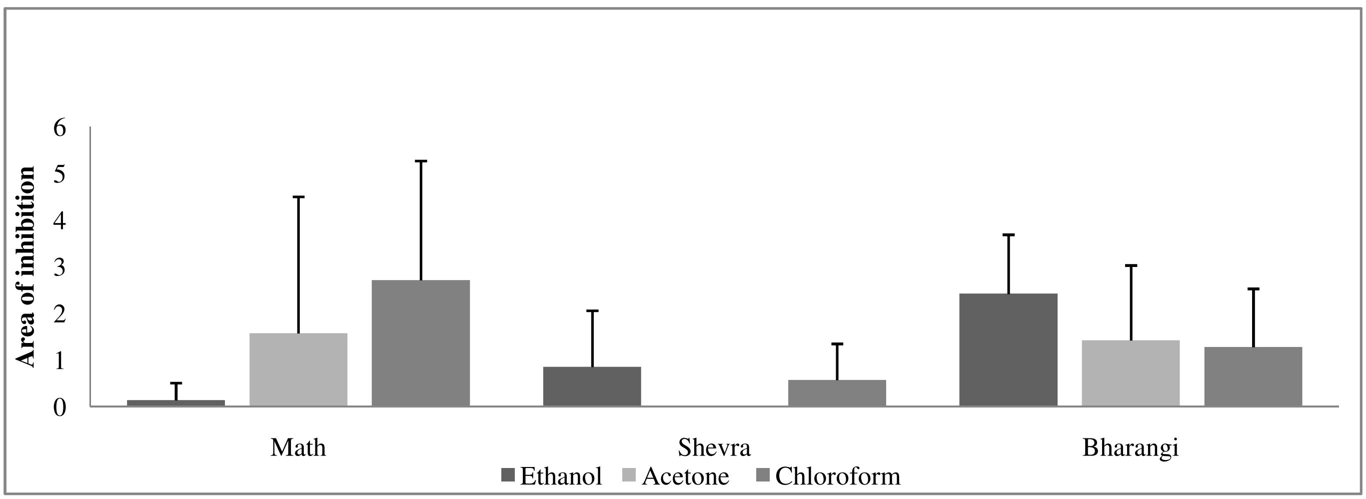


**Figure-2**
**Effect of different solvents extracted from Seasonal vegetables on S. aureus growth**

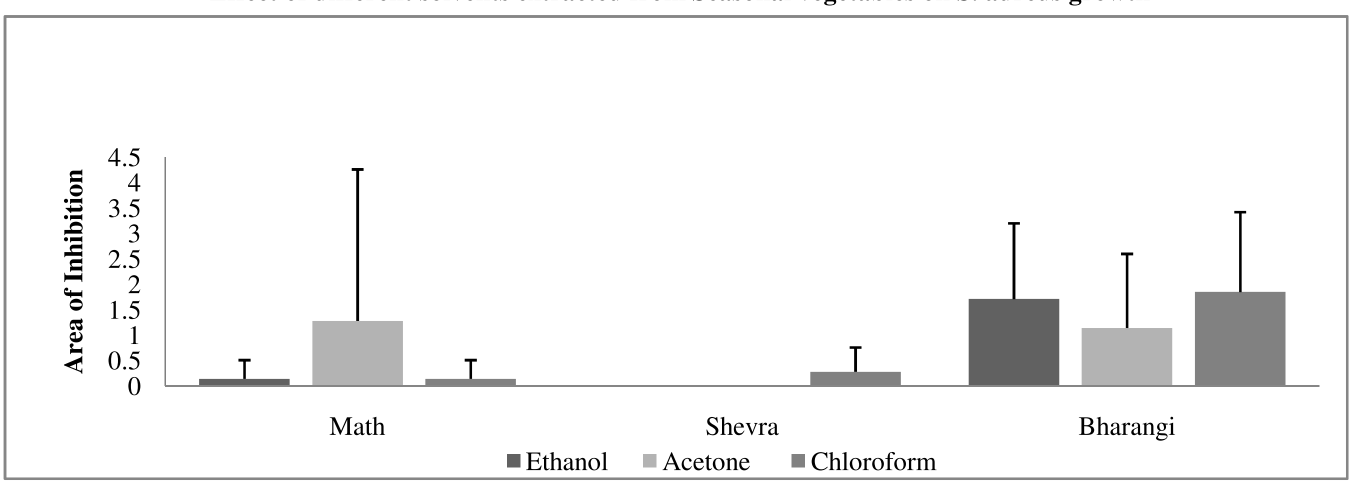


**Figure-3**
**Effect of different solvents extracted from Seasonal vegetables on K.pneumoniae growth**

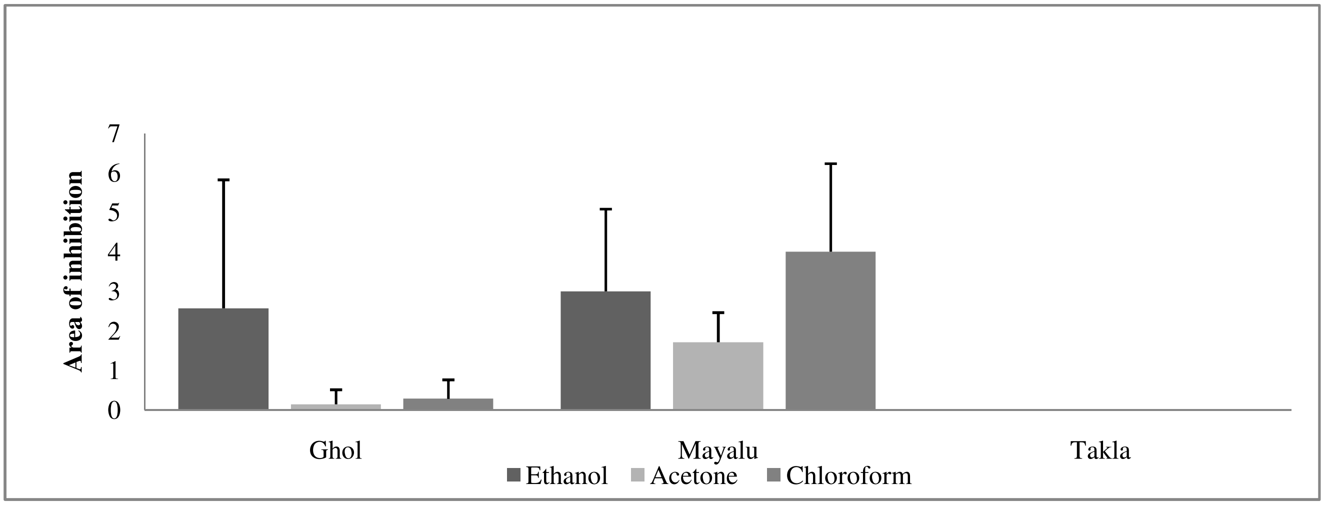


**Figure-4**
**Effect of different solvents extracted from All Seasonal vegetables on E.coli growth**

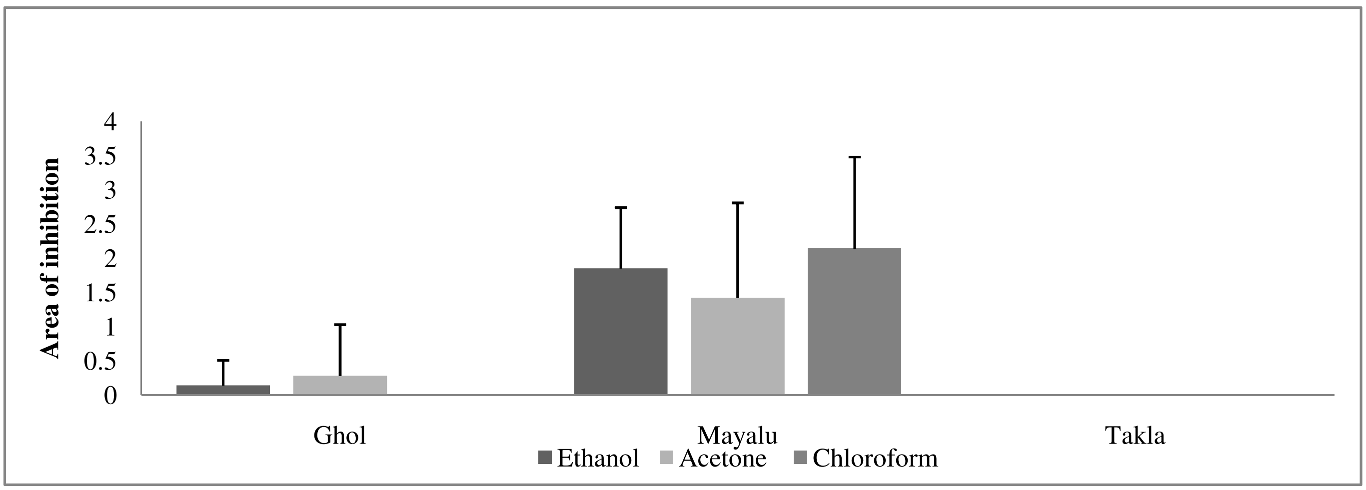


**Figure-5**
**Effect of different solvents extracted from All Seasonal vegetables on S.aureus growth**

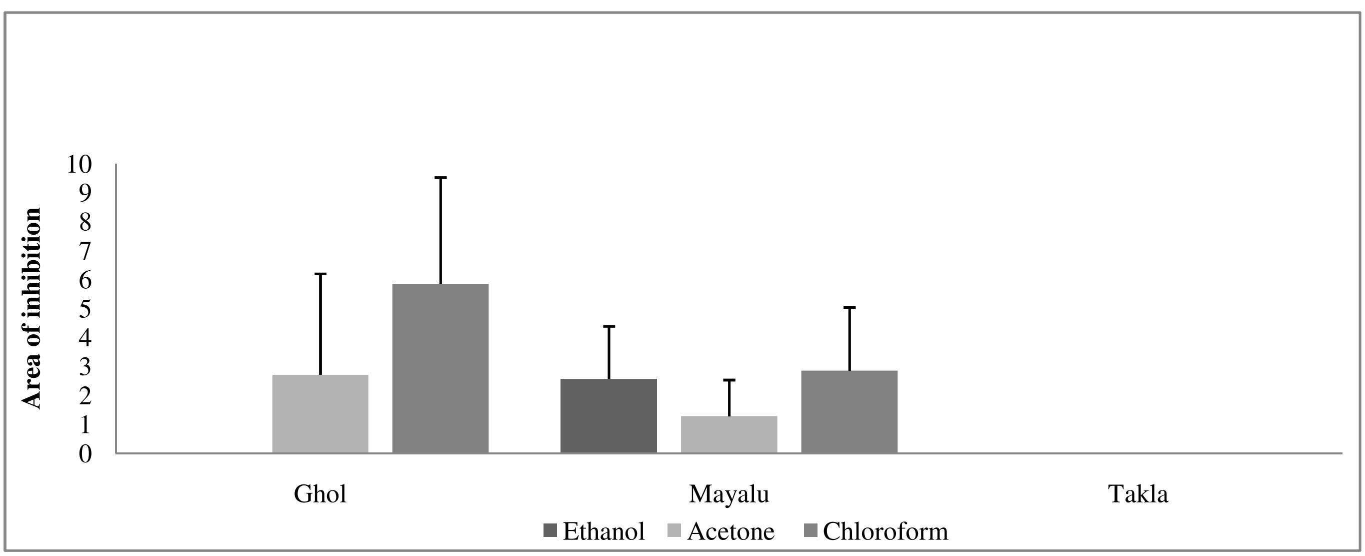


**Figure-6**
**Effect of different solvents extracted from All Seasonal vegetables on K.pneumoniae growth**

## Conclusion

The solvent extracts i.e. ethanol, acetone and chloroform of the monsoon plants have shown better activity against all three microorganisms. The ethanolic extract of Bharangi plant was found to be best against *E.coli* while chloroform extract of Math was best against *K.pneumoniae* while the ethanol extract of Shevra plant was effective only against *S.aureus* and *K.pneumoniae* while the acetone extract was not at all effective against all the three test organisms.

As compared to all season plants the solvents extracts i.e. ethanol, acetone and chloroform also showed good antibacterial activity against all the three organisms used with the exception of Takla plant which did not showed any inhibition in all the three solvents against the test organism used.

**Discussions:** Medicinal plants have provided a source of inspiration for novel drugs compounds since plant derived medicines have made large contributions to human health. The traditional healers make use of water primarily as solvent but extracts of these plants were certainly much better and powerful. This may be due to the better solubility of the active components in organic solvents[36].

The bioactivity of plant extracts was attributed to phytochemical constituents. In the present study, crude extracts of the plant material obtained in ethanol, acetone & chloroform solvents were tested against three commonly occurring bacteria *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*. These extracts exhibited that they could inhibit the growth of both, Gram -positive and Gram-negative bacteria; suggesting their inhibitory effect on broad spectrum of pathogenic microorganisms. However, the variation in the size of zone of inhibition indicates, the varying degree of efficacy of different phytoconstituents of the plant used, on the target organism. Also, it will be interesting to study how each solvent can preserve the antibacterial properties of the plants. Further studies are required to isolate, identify and elucidate the structure of the bioactive compound. It should also be noted that active compounds present in the crude plant extracts showed the antibacterial activity in a dose dependant manner. If the concentration of the active ingredient/component is increased, there could be other constituents exerting antagonistic effects on the bioactive compounds of our interest. So, use of the crude extracts of these plant samples as an agent to control microbial pathogens needs further extensive research for their better economic and therapeutic utilization. Nevertheless, all the five plant samples used in this research work could be looked at as a potential source of antibacterial agents from natural sources.

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