Comparative Analysis of Antimicrobial Activity of Herbal Extracts against Pathogenic Microbes

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Abstract

Antimicrobial activities of Spinacia oleracea leaf, Zingiber officinalis rhizome, Coriandrum sativum leaf, Allium sativum clove, Aloe vera gel and leaf was carried out against the multi-drug resistant strains using the minimum inhibitory concentration method. The direct TLC bioautography method is performed to identify the bioactive compounds present in the extracts exhibiting the antimicrobial property. All the plant extracts effectively inhibited the growth of pathogenic strains used in the study at a concentration of 250 to 31.25 mg/ml. These results provide evidence that the tested plant extracts possess antimicrobial properties which can be tested further in the development of novel antimicrobial agents.

Keywords: Antimicrobial activity; TLC bioautography method; Herbal extracts; multi-drug resistant strains; Mcfarland

Abbreviations: MIC: Minimum Inhibitory Concentration; DMSO: Dimethyl Sulfoxide; TLC: Thin Layer Chromatography; NCIM: National Collection of Industrial Microorganisms; CFU: Colony Forming Units

Introduction

The emergence of multi-drug resistant microorganisms with decreased susceptibility to antibiotics due to improper usage of broad spectrum drugs is increasing day by day globally. In developing countries, the increase in the rate of mortality and morbidity is due to the infectious diseases [1, 2]. Synthetic drugs which are used to treat infectious diseases are expensive and often cause side effects. Hence, there is a need to develop novel drugs to control the spread of pathogenic microbial strains. Plants with medicinal value are of interest as they are the richest source of secondary metabolites with therapeutic properties considered to be safe and effective compared to synthetic drugs [3]. The different types of plant material like leaves, root, stem, flowers, fruits and vegetables are potential sources of antimicrobial compounds due to the presence of bioactive compounds. Medicinal plants contain large number of secondary phytomedicines like phenols, flavonoids, alkaloids, saponins, tannins, steroids etc. possess synergistic effects used for remedial purposes [4]. These secondary bioactive compounds have different structures with different mode of action used to control microbial growth and survival. Therefore, many dreadful diseases can be cured with the use of plant preparations [5]. The importance of natural products obtained from plants remains unchanged even after the developments in the field of synthetic organic chemistry and pharmacology. They have been used widely as traditional medicine and particularly when synthetic drugs are inaccessible and unaffordable [6]. A large number of plant species were screened for their antimicrobial properties by many researchers till now. Since ancient times, herbs and spices have been used as flavoring agents, food additives and as preservatives. They are effective in eradicating the microbial population, used widely in food industries generally considered as safe. The antimicrobial activity varies depending upon the type of food, type of spice, microorganism, essential oils and type of extracts used [7]. The study of bioactive compounds and the development of plant based antimicrobial drugs have gained importance in current research due to increase in demand for safe and effective drugs.

The present study mainly focused on Aloe vera gel, leaf, Spinacia oleracea leaf, Zingiber officinalis rhizome, Coriandrum sativum leaf and on an Allium sativum clove to evaluate their antimicrobial properties which could provide valuable information in order to use them as therapeutic tools for control of microbial growth. The extracts were prepared using solvents of different polarities and the antimicrobial activity was tested against multi-drug resistant strains of Escherichia coli, Candida albicans, Enterococcus faecalis, Staphylococcus aureus, Streptococcus mutans, Klebsiella pneumoniae and Mycobacterium smegmatis using MIC method. The direct TLC bioautography method is used as a tool to detect and isolate the antimicrobial compounds from plant extracts directly even in small amounts and is considered to be a good method compared to agar dilution methods. Aloe vera is a succulent plant well known for its medicinal properties belongs to the family of "Aloaceae". It is a stemless plant grows up to a...
height of 60-100cm and the leaves are thick and fleshy [8]. Poly-
saccharides, monosaccharides, chromones, anthraquinones, or-
ganic compounds, inorganic compounds, vitamins and enzymes
are some of the active compounds present in Aloe vera were
responsible for antibacterial, antifungal, antioxidant, wound healing
and anti-diabetic properties [9,10].

The antimicrobial effects of Aloe vera have been attributed
to the plant's natural anthraquinones capable of inhibiting the
strains of Mycobacterium tuberculosis and Bacillus subtilis. The
thick, colorless mucilaginous gel material present within the
leaves of Aloe vera is considered to be effective in curing gastro-
intestinal diseases [11], burns and wounds [12]. It is also used to
boost up the immune system in humans [13] and the products
obtained from Aloe vera are used widely in cosmetic, food and
pharmaceutical industries [14]. Coriandrum sativum also known
dhania is an annual herb belongs to the family of “Apiaceae”. It
grows up to a height of 50cm. The leaves are variable in shape,
broadly lobed at the base of the plant. It is used to treat diarrhea,
vomiting, rheumatism, cough, dysentery, joint pains and indiges-
tion [15]. It possesses anti-diabetic, anti-inflammatory, lipolytic,
antihypertensive, nervel axing and antiseptic properties. The
coriander extracts possess free radical scavenging, antioxidant,
antimutagenic, anticancerous and antibacterial properties [16].
Allium sativum commonly called as garlic is a bulbous plantbel-
ongs to the family of “Amaryllidaceae”. It has good anti-bacterial
and anti-fungal properties. Garlic is used to prevent high blood
pressure, high cholesterol and heart diseases, provides protection
against cancer [17]. Spinacia oleracea commonly known as Palak/
spinach used widely in India is an edible flowering plant belongs
to the family of “Chenopodiaceae”. It is used to treat leprosy, asth-
ma, urinary diseases, lung inflammation, joint pains, thirst, sore
throat, scabies, vomiting, ringworm, sore eye, cold, sneezing, fever
and the diseases related to brain and heart [18]. Spinach is rich in
vitamin A, E, C and K, foliate, iron, magnesium and manganese. It
also possesses several biological activities like antimicrobial, anti-
viral, anticancer, antioxidant and anthelmintic properties. Zingib-
ero officinale commonly called as ginger is an herbaceous perennial
flowering plant belongs to the family of “Zingiberaceae”. Ginger is
widely used as a spice possesses medicinal value. It is used to treat
asthma, nausea, colic, cough, cold, rheumatism, loss of appetite,
swelling, heart diseases and dyspepsia [19].

Materials and Methods

Collection of plant material

The plant materials considered for the present are Aloe vera,
Spinacia oleracea, Zingiber officinale, Coriandrum sativum and Al-
lium sativum which were collected from the local market of Vi-
sakhapatnam.

Microorganisms used

The microbial strains considered for the present study are Escherichia coli, Staphylococcus aureus, Enterococcus faecalis,

Streptococcus mutans, Candida albicans, Mycobacterium smegma-
tis and Klebsiella pneumonia. All the strains were obtained from
the National Collection of Industrial Microorganisms (NCIM),
Pune, Maharashtra, India.

Preparation of extracts

Aloe vera leaves, Spinacia oleracea leaf, Zingiber officinale rhiz-
zone, Coriandrum sativum leaves and Allium sativum coves were
washed thoroughly under running tap water and then with distilled
water to remove dirt. They were then air-dried under shade, 
away from sunlight for 4-5 days and made into a fine powder
using mortar and pestle. Extracts were prepared using the sol-
vent methanol, ethyl acetate, petroleum ether and chloroform at
a concentration of 1:10 ratio. The extraction is carried out with
vigorous shaking for 48-72h followed by filtration. The extract is
then concentrated using Rota evaporator and is further diluted to
required concentration using DMSO just before use.

Inoculum preparation

UTI agar medium is used to culture the strains of Escherichia coli and Enterococcus faeal. Mannitol Salt agar medium is used for the growth of Staphylococcus aureus. The Streptococcus Selection medium used for Streptococcus mutans and Rose Bengal medium used for culturing Candida albicans strains respectively. The agar slants with respective cultures were prepared and incubated at 37°C for 24h. The colonies of test organisms grown over-
night were inoculated into 0.85% normal saline and the turbidity
adjusted to 0.5 Mcfarland using the standard which is equal to
1.5×10^5 CFU/mL. It was further diluted to obtain the final inoculum
of 5×10^5 CFU/mL.

Antimicrobial assay using MIC method

MIC was performed as per Clinical and Laboratory Standards
Institute guidelines using extracts against bacterial and fungal pathogens in a 96 well u-bottomed microtitre plates. The plant ex-
tracts were serially diluted from the concentration of 500 mg/ml to
0.02 mg/ml and then added with the final inoculum of 5×10^5 CFU/
ml. The anti-microbial compound and the final inoculum were in
the ratio of 1:1 (v/v). Each test performed in triplicate with posi-
tive and negative controls. After the addition of inoculum, plates
were sealed with aluminum foil and incubated at 37°C for 24h in
the case of bacterial cultures and for 48 h at 28°C for fungal cul-
tures respectively in an incubator. At the end of incubation period,
the wells were added with 20 ul of 0.1 mg/ml resazurin dye and
incubated for 30min in the color development. Presence of bac-
terial or fungal growth is indicated by a change in the color of the
medium to pink, whereas no color change indicates the absence
of growth of the organism and the least concentration where there
is no growth is considered as an MIC value of that particular compo-
und against bacterial and fungal strains used. The experiment
is carried out in triplicate using the standards streptomycin, fluco-
nazole, cefixime and rifampicin.

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Thin layer chromatography

Thin layer chromatography (TLC) was performed using 0.2mm silica coated aluminum sheets purchased from Merck. The mobile phase of chloroform: methanol in the ratio of 9:1 is used for the separation of bioactive compounds present in the plant extracts. 10µl of each extract was spotted separately on the TLC sheet, allowed for the migration of compounds. After complete elution; the spots were identified and calculated \( R_f \) values for each band.

\[
R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}
\]

Direct TLC bioautography method

Bioautography is a rapid analytical technique used in the identification of bioactive lead/ scaffolds in complex matrices of plant extracts. In this method of direct TLC Bioautography, the developed TLC plate was sprayed with fungal and bacterial suspensions incubated at 25°C for 48h under humid conditions. After the incubation period, resazurin dye was sprayed and again incubated at 37°C for 3 to 4h. The antimicrobial activity is indicated by the appearance of clear white zones against a purple background on the TLC plate.

Results and Discussion

Antimicrobial assay

The antibacterial and antifungal study is carried out against the strains of *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus mutans*, *Candida albicans*, *Mycobacterium smegmatis* and *Klebsiella pneumonia*. Using the extracts of *Aloe vera*, *Spinacia oleracea*, *Zingiber officinale*, *Coriandrum sativum* and *Allium sativum*. All the extracts showed antimicrobial activity against the tested pathogenic strains and the results were shown in (Table 1). The petroleum ether and methanolic extracts of *Aloe vera* and *Coriandrum sativum* is effective against *M. smegmatis* compared with other strains. The extracts prepared with solvents of chloroform, methanol, petroleum ether and ethyl acetate showed antimicrobial activity at a concentration ranging from 250-31.25mg/ml respectively against all the tested strains. *Spinacia oleracea* and *Zingiber officinale* petroleum ether, chloroform and ethyl acetate extracts showed effective inhibitory activity against the strains of *M. smegmatis* and *K. pneumonia* at a concentration of 31.25mg/ml, whereas, the MIC value ranged from 250-3.125mg/ml against the other strains used in the study. *Candida albicans* and *Klebsiella pneumonia* were inhibited at a concentration of 31.25mg/ml with *Allium sativum* methanol and chloroform extracts.

| Plant Species | Strain Name | Chloroform | Methanol | Petroleum ether | Ethyl acetate |
|---------------|-------------|------------|----------|----------------|--------------|
| *Aloe vera* leaf | *E. coli* | 250 | 250 | 250 | 250 |
| | *S. aureus* | 250 | 250 | 125 | 250 |
| | *E. faecalis* | 250 | 250 | 125 | 250 |
| | *S. mutans* | 250 | 250 | 250 | 250 |
| | *C. albicans* | 62.5 | 62.5 | 125 | 125 |
| | *M. smegmatis* | 62.5 | 31.25 | 62.5 | 125 |
| | *K. pneumonia* | 250 | 125 | 250 | 125 |
| *Aloe vera* gel | *E. coli* | 125 | 125 | 250 | 250 |
| | *S. aureus* | 125 | 250 | 250 | 250 |
| | *E. faecalis* | 125 | 125 | 250 | 250 |
| | *S. mutans* | 125 | 250 | 250 | 125 |
| | *C. albicans* | 62.5 | 125 | 125 | 62.5 |
| | *M. smegmatis* | 62.5 | 31.25 | 31.25 | 62.5 |
| | *K. pneumonia* | 31.25 | 62.5 | 125 | 62.5 |
| *Spinacia oleracea* leaf | *E. coli* | 125 | 250 | 250 | 250 |
| | *S. aureus* | 250 | 250 | 250 | 125 |
| | *E. faecalis* | 125 | 250 | 250 | 125 |
| | *S. mutans* | 250 | 250 | 250 | 250 |
| | *C. albicans* | 125 | 125 | 62.5 | 62.5 |
| | *M. smegmatis* | 62.5 | 31.25 | 31.25 | 62.5 |
| | *K. pneumonia* | 125 | 62.5 | 125 | 62.5 |
From the above study, it was observed that all the extracts showed potent antimicrobial activity against all the screened bacterial and fungal strains. The plant extracts effectively inhibited the growth of tested pathogenic strains in the order of *Mycobacterium smegmatis* > *Klebsiella pneumoniae* > *Candida albicans* > *Enterococcus faecalis* > *Staphylococcus aureus* > *Escherichia coli* > *Streptococcus mutans* and the inhibitory effect of plant extracts was in the order of *Spinacia oleracea* leaf > *Aloe vera* leaf > *Aloe vera* gel > *Coriandrum sativum* leaf > *Allium sativum* cloves > *Zingiber officinalis* rhizome. The methanol extracts showed higher antimicrobial activity followed by chloroform, petroleum ether and ethyl acetate. The polarities of solvents play a vital role in the extraction of plant secondary metabolites that influence the antimicrobial potential of extracts [20]. The toxicity of solvent, handling of extracts, total yield and the duration of extraction are some of the important factors need to be considered to increase the extraction efficiency in order to obtain maximum product out of the plant material used [21].

The antimicrobial activity of extracts could be due to the presence of several plant bioactive compounds. The method of preparation and the type of solvent influence the antimicrobial potential of plants and the variations in the activity of tested plant species is due to the difference in the composition of phytochemicals. Antibiotics were most commonly used to kill the microbes and protect patients from infectious diseases, but there is an increased emergence of multi drug resistant strains that leads to treatment failure. Microbes acquire resistance to antibiotics preventing destruction by means of intrinsic mechanism [22]. To control human health problems associated with increasing population day by day, there is a need to discover new antimicrobial drugs for combating the growth of harmful microbes. Drugs with therapeutic properties obtained naturally from the source of medicinal plants are of great importance as these natural plant products are likely to be effective against multi drug resistant strains. Secondary metabolites produced by plants are useful in treating diseases like diabetes, heart diseases, infectious diseases and cancer [23]. It was reported that the garlic has an anti-infective property and antimicrobial activity against a large number of microbes including viruses. Allicin primarily inhibits the synthesis of DNA, RNA and proteins, which is an active ingredient of garlic [24]. The antimicrobial activity of garlic is due to the presence of phenolic compounds and organosulfur compounds [25]. Similar work supporting our results of antimicrobial activity of *Aloe vera* gel against the strains of *E. coli* and *S. aureus* was also reported [26]. The antimicrobial activity of *Aloe vera* juice against the strains of *Mycobacterium smegmatis*, *S. aureus*, *Enterococcus faecalis*, *M. luteus*, *B. sphericus*, *Paeruginosa*, *K. pneumoniae*, *E. coli*, *S. typhimurium* and *Candida albicans* was reported [27]. The inhibitory effect of *Aloe vera* juice against the strains of *M. smegmatis*, *K. pneumoniae*, *E. faecalis*, *M. luteus*, *C. albicans* and *B. sphericus* were also reported [28]. Caffeic acid, chlorogenic acid, ferulic acid, flavonols like quercetin, protocatechuic acid and other polyphenols are present in the leaf extracts of fresh coriander [29]. Due to the presence of polyphenols along with quercetin, coriander is used in food and pharmaceutical industries to avoid the bacterial contamination. The antimicrobial and antioxidant potential of polyphenols pres-

| Zingiber officinalis rhizome | E. coli | 62.5 | 125 | 125 | 62.5 |
|-----------------------------|--------|------|------|------|------|
|                             | S. aureus | 250  | 125  | 250  | 125  |
|                             | E. faecalis | 125  | 125  | 125  | 250  |
|                             | S. mutans | 125  | 250  | 125  | 125  |
|                             | C. albicans | 125  | 62.5 | 250  | 250  |
|                             | M. smegmatis | 125  | 62.5 | 31.25 | 62.5 |
|                             | K. pneumonia | 62.5 | 31.25 | 250  | 31.25 |

| Coriandrum sativum leaf | E. coli | 125 | 250 | 125 | 125 |
|-------------------------|--------|------|------|------|------|
|                         | S. aureus | 250  | 250  | 250  | 125  |
|                         | E. faecalis | 125  | 250  | 250  | 250  |
|                         | S. mutans | 250  | 250  | 250  | 250  |
|                         | C. albicans | 125  | 125  | 62.5 | 62.5 |
|                         | M. smegmatis | 62.5 | 31.25 | 31.25 | 62.5 |
|                         | K. pneumonia | 125  | 62.5 | 125  | 62.5 |

| Allium sativum cloves | E. coli | 250 | 250 | 250 | 250 |
|-----------------------|--------|------|------|------|------|
|                       | S. aureus | 125  | 250  | 250  | 250  |
|                       | E. faecalis | 125  | 125  | 125  | 250  |
|                       | S. mutans | 250  | 125  | 125  | 250  |
|                       | C. albicans | 62.5 | 31.25 | 125  | 62.5 |
|                       | M. smegmatis | 125  | 125  | 62.5 | 125 |
|                       | K. pneumonia | 31.25 | 62.5 | 250  | 62.5 |
ent in *Coriandri*ts effective against the strains of *E. coli*, *P. aerugino-
sa* responsible for urinary tract infections and gastroenteritis and against *S. aureus*, causative organism of pneumonia, toxic shock syndrome and food poisoning. Ginger rhizome contains gingerol and shaghol active compounds along with several bioactive com-
ounds exhibiting a broad range of antimicrobial activity. The inhibitory effect of the ginger ethanol extract against candida albicans was reported [30]. Recent studies revealed that zingerone showed protective action against *E. coli* responsible for diarrhea. Study shows that the methanol extracts of ginger effectively inhibited the growth of *E. coli* and *S. aureus* similar to our study [31].

The inhibition of bacterial strains *S. typhimurium*, *E. coli*, *P. multocida*, *M. luteus*, *L. bulgaricus*, *S. aureus*, *K. pneumoniae*, *P. vulgaris* and *S. epidermis* by *S. oleracea* was reported [32]. Alkoldoids, tan-
nins, steroids, glycosides and terpenoids are some of the phenolic

**TLC separation of bioactive compounds**

Thin layer chromatography is performed to identify the num-
ber of bioactive compounds present within each extract. The extrac-
ts prepared using methanol, ethyl acetate and petroleum ether showed 5-6 distinct bands whereas, chloroform extracts of *Coriandrum sativum* leaf, Spinach leaf, *Aloe vera* leaf and *Zingiber officinalis* rhizome showed 6, 8, 5 and 6 distinct bands respectively. All the extracts used in the study gave impressive results which di-
rectly represents the presence of phytochemicals. It was observed that the *Spinacia oleracea* leaf extract possesses a large number of Phyto-constituents compared to others which could be the reason of higher antimicrobial activity.

The separation of the bio active compounds present within the plant extracts is achieved using the technique of thin layer chromatography, revealed the presence of several phytochemicals with different R<sub>v</sub>values. The suitable mobile phase can be selected based on the R<sub>v</sub>values of each compound. The polarity of the compounds can be detected using the R<sub>v</sub>values. The technique of TLC gives idea for the selection of appropriate solvent system for separation of compounds in pure form for further studies.

**Direct TLC bioautography method**

The method of direct TLC bioautography is used to detect the compounds with antimicrobial potential present within the plant extracts. Effective inhibition of the tested plant extracts against pathogenic strains was in the order of *Mycobacterium smeg-
matis* > *Klebsiella pneumoniae* > *Candida albicans* > *Enterococcus faecalis* > *Staphylococcus aureus* > *Escherichia coli* > *Streptococcus mutans*. All the plant extracts used were found to be most effective against *Mycobacterium smegmatis*and*Klebsiella pneumoniae* showing clear white zones against a purple background on the TLC plate indicating the antimicrobial activity.

Plant extracts contain a large number of bioactive compounds and thus the screening of compounds exhibiting the antimicrobial activity is necessary which is made easier with the use of the TLC bioautography method. The zones of inhibition with creamy spots against purple background are visualized after spraying the plates with resazurin dye. This method is considered to be convenient for obtaining the reliable information on the activity of single compounds. Study of herbal extracts suggests the use of herbal preparations for preservation of foods, curing infectious diseases caused by pathogenic microbes and to prevent the microbial deterioration of food products. The isolation and characterization of active compounds with biological activities of these herbal plants could be used to generate a novel drug for future prospects.

**Conclusion**

From the above study, it can be concluded that all the plant extracts showed promising antimicrobial activity against harmful pathogenic strains. Extracts exhibiting variations in the antimicrobial activity is due to differences in the composition of bioactive compounds extracted depending upon the polarity of the solvent. Further research is going on in this area to isolate and characterize the bioactive compounds of *Aloe vera*, *Coriandrum sativum*, *Allium sativum* and *Zingiber officinalis*, which may provide a scope of developing more effective drugs for combating infectious diseases.

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**Conflict of Interest**

No conflict of interest declared by authors.

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