INTRODUCTION

Life and ailment are inseparable. They go together. Almost they can be described as the two sides of a coin. Drugs are used to cure ailments, diseases or disorders. But persistent use of some drugs, particularly for certain long-standing diseases poses some problems. Development of drug resistance is one such problem. Often it may be observed that drug resistance is associated with the usage of antibiotics.

This has made the scientists and researchers to take up the challenge of finding out antimicrobial substances from other source. Plants are the best source for main needs like food, clothing, and shelter and so on. Now the researcher has turned towards the plants for such anti-microbial substances too. Plants are known to produce a variety of compounds to protect themselves against a variety of their pathogens and they are considered as a potential source for different classes of antimicrobial substances [1-4].

Plants that are used in traditional medicine contain a wide range of substances which can be used in the treatment of chronic as well as acute infectious diseases. The substances that can either inhibit the growth of micro-organisms or kill them are considered the candidates for developing new drugs for treatment of various infectious diseases.

The plants used in traditional system of medicine are well known as remedies for diseases in the rural areas of developing countries [5-8]. Herbal medicines have been used in developing countries as an alternative to Allopathic medicines.

The extensive review of literature take up in the earlier chapters has revealed a variety of medicinal plants possessing anti-inflammatory activity which also exhibited good antimicrobial properties [9, 10, 11]. On the basis of the above reports and since selected plants in our study possessing anti-inflammatory activity, the plant extracts were subjected to study the antimicrobial activity against various bacterial and fungal strains.

The term microbiological assay is a biological assay performed with microorganisms like bacteria, yeast, moulds etc. This involves the measurement of the relative potency or activity of compounds by determining the...
amount of test material required for producing stipulated
effect on suitable organism under standard conditions.

2. General methods for Anti-microbial
There are number of methods available to
evaluate the antimicrobial activity. They are,
A) Diffusion method
B) Dilution agar method
C) Cup-plate method
From the above methods we used cup-plate method.

2.1 Antibacterial Activity of BRL & BRS Extracts
by Cup-Plate Method
2.1.1 Bacteria used
a) Pseudomonas.Aeruginosa
b) Bacillus.Subtitis

2.1.2 Standardization of Micro-Organisms:
• One loop-full of micro-organisms were inoculated into
100 ml of sterile medium and incubated for 24 h at 37 °C
for bacterial culture. After 24/48 hours of incubation, 1 ml
of broth containing the micro-organisms was added to 9
ml of peptone water.
• 10 fold serial dilutions were made in the range of 10⁻¹
to 10⁻⁸. 100 µl of the dilutions ranging from 10⁻⁵ to 10⁻⁸
were spread over the sterile nutrient agar (SNA) plates
and kept at 37 and 27 °C for 24/48 hours respectively.
The number of colony forming units (CFU) was
counted and number of micro-organisms per 1 ml of stock
culture was calculated.

2.1.3 Preparation of test and standard solutions:
The stock solution of test compounds was
prepared by dissolving the dried extracts at a
concentration of 5µg/ml, 10µg/ml, and 15µg/ml in
dimethylsulphoxide (DMSO) respectively. The stock
solutions of reference standards (Chloramphenicol and
Nystatin) were prepared at a concentration of 0.6 µg/ml in
sterile water.
Antimicrobial activity was screened by adding
0.05 ml stock solution to each cup by micropipette.

2.1.4 Culture Medium:
The following media were used for the present
antimicrobial studies.

2.1.5 Nutrient agar for bacteria:
Beef extract-0.3%
Sodium chloride -0.5%
Peptone-0.5%
Agar-2.0%
PH-7.2-7.4
The sterilized medium was cooled to 40 °C and poured
into the petri dishes to contain 6 mm thickness. The media
was allowed to solidify at room temperature.

2.1.6 Sterilization:
Sterilization of the media, water etc., were carried
out by autoclaving at 15 lbs/inch² for 20 minutes. The
glassware like syringes, petridishes, pipettes, empty test
tubes were sterilized by dry heat in an oven at a
temperature of 160 °C for one hour.

2.2 Procedure:
2.2.1 Determination of zone of inhibition by cup
plate method (Indian Pharmacopoeia., 1996):
The cylinder plate assay of drug potency is based
on measurement of the diameter of zone of inhibition of
microbial growth surrounding cylinders (cups), containing
various dilutions of test compounds. A sterile borer was
used to prepare four cups of 6 mm diameter in the agar
medium spread with the micro-organisms and 0.1 ml of inculums. These cups were spread on the agar plate by
spread plate technique. Accurately measured (0.05 ml)
solution of each concentration and reference standards
were added to the cups with a micropipette.

All the plates were kept in a refrigerator at 2 to 8
°C for a period of 2 hours for effective diffusion of test
compounds and standards. Later, they were incubated at
37 °C for 24 hours. The presence of definite zone of
inhibition of any size around the cup indicated
antibacterial activity. The solvent control was run
simultaneously to assess the activity of dimethyl
sulphoxide and water which were used as a vehicle. The
experiments were performed three times. The diameter of
the zone of inhibition was measured and recorded.
2.3 Results and Discussion:

**Table No-1: Effect of BRL & BRS Extracts of Anti-Bacterial Activity**

<table>
<thead>
<tr>
<th>Plant Extract</th>
<th>Conc. µg/ml</th>
<th>Anti-Bacterial Activity Zone Of Inhibition (Mm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gram(+Ve)Pathogenic Bacteria (B. Subtilis)</td>
<td>Gram(-Ve)Pathogenic Bacteria (P. Aeruginosa)</td>
</tr>
<tr>
<td>Alcoholic extract of Leaf</td>
<td>15µg/ml</td>
<td>2.1cm</td>
<td>2.3cm</td>
</tr>
<tr>
<td></td>
<td>10µg/ml</td>
<td>1.8cm</td>
<td>2.2cm</td>
</tr>
<tr>
<td></td>
<td>5 µg/ml</td>
<td>1.5cm</td>
<td>2cm</td>
</tr>
<tr>
<td></td>
<td>Standard</td>
<td>0.9 cm</td>
<td>0.7 cm</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Alcoholic extract of Stem</td>
<td>15µg/ml</td>
<td>1.4 cm</td>
<td>0.9 cm</td>
</tr>
<tr>
<td></td>
<td>10µg/ml</td>
<td>0.8cm</td>
<td>0.7cm</td>
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<tr>
<td></td>
<td>5 µg/ml</td>
<td>0.4cm</td>
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<td>0.9 cm</td>
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<tr>
<td></td>
<td>Control</td>
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<td>_</td>
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<tr>
<td>Chloroform extract of Leaf</td>
<td>15µg/ml</td>
<td>0.8cm</td>
<td>0.7cm</td>
</tr>
<tr>
<td></td>
<td>10µg/ml</td>
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<td>0.1cm</td>
</tr>
<tr>
<td></td>
<td>Standard</td>
<td>0.7 cm</td>
<td>0.6 cm</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>control</td>
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<td>_</td>
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</tbody>
</table>

The in-vitro anti-bacterial activity using cup plate method was tested with different concentrations of BRL & BRS was shown in Table No: 1. The in-vitro anti-bacterial activity using cup-plate method. Anti-bacterial activity was carried out on four bacterial strains like Pseudomonas Aeruginosa and Bacillus Subtilis, BRLE extract showed significant activity against microbes at the concentrations of 5µg/ml, 10µg/ml, and 15µg/ml. when compared with that of Amphicillin act as standard.

In the maximum activity was obtained when BRLAE & BRSAE at concentration of 15µg/ml, 10µg/ml, 5 µg/ml against the two micro-organism (2.2 & 2.1 mm) respectively. Where BRLCE & BRSCE at concentration of
15µg/ml, 10µg/ml, 5 µg/ml against the two microorganisms (0.8 & 0.7 mm) respectively showed minimum activity.

It was found that the BRLAE & BRSAE contains tannis, phenolic compounds and alkaloids, among them methyl tiglate in the ethanolic extracts may be responsible for anti-bacterial activity.

The anti-bacterial activity of BRLAE at 15µg/ml is more significant than that of BRSAE at 15µg/ml. The anti-bacterial activity of BRLE is more significant than that of BRSE.

References

Competing Interests:
The authors declare that they have no competing interests.

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