CHAPTER - IV

STUDIES ON THE ANTIMICROBIAL EFFICACY OF
ESSENTIAL OILS
STUDIES ON THE ANTIMICROBIAL EFFICACY OF ESSENTIAL OILS.

INTRODUCTION:

The effect of essential oils in destroying or inactivating micro-organisms has received great attention in recent years from research workers. The recognition and study of powerful germicidal action of essential oils stimulated interest for studying the possible bactericidal potentials.

Antiseptic properties of the essential oils are being investigated to a large extent. Although the bacterial origin of infection was unknown before the work of Pasteur and Lister, disinfectants have been used empirically from the earliest times.

The ancient Egyptians used them in the embalming of bodies. The burning of aromatic substances and sulphur was used to combat the spread of the bubonic plague in the Middle Ages.

Many terms have been used to describe disinfectants e.g., germicide, antiseptic, bactericide etc. During recent years these terms have been given more precise interpretations. A disinfectant is a substance that kills pathogeni
(disease producing) organisms and thus prevents infection. Germicide is usually accepted as synonymous.

**Bactericide**⁴: This has a restricted meaning being an agent that kills bacteria.

**Fungicide**⁵: A fungicide kills fungi, yeast and similar micro-organisms.

**Antiseptic**⁶: This term is often wrongly used as a synonym for disinfectant. Antiseptic is a substance that prevents the multiplication of bacteria.

**Bacteriostatic**⁷: A bacteriostatic by definition inhibits the growth of bacteria but does not kill them and similarly a fungistatic inhibits the growth of fungi, yeast and similar micro-organisms.

Essential oils differing so widely in their source and chemical composition must manifestly exercise different uses. There are certain properties common to many of the essential oils. Essential oils are well known for their scenting and flavouring properties and are widely used in consumer products and in medicine. Many essential oils are powerful germicides and nearly all of them are more or less antiseptics.⁸ A comparative antiseptic activity of the important constituents of the essential oils was studied against the typhoid bacillus by Ridal and Miller⁹ who observed activities in the following order.
Anethole 1
Cinnamaldehyde 5
Citral 5
Eucalyptol 2
Eugenol 9
Geraniol 7
Menthol (natural) 5
Methyl salicylate 2
Safrol 1
Santalol 1
Thymol 25

Myers\textsuperscript{10} also reported on the fungicidal action of the essential oils and some related components which are not always parallel to their bactericidal power. He found the following to be potent (in order of their efficiency):

Thymol, carvacrol, mustard oil, cinnamon oil, clove oil, turpentine oil, eucalyptol oil, camphor, menthol, methyl salicylate and anisi oil.

Chamberland\textsuperscript{11} studied on Anthrax bacilli contained in blood with essential oils and found that the oil of Vespetro - 18 hours, oil of Angelica - 40 hours and oil of Cyloncinnamom - 65 hours, can kill the bacilli at 37°C.

Penfold and Grant\textsuperscript{12} and Rideal\textsuperscript{13} systematically studied the disinfecting properties of Australian essential oils and their constituents. De and Subrahmanyan\textsuperscript{14} have determined the germicidal values of Indian oils and of their constituents. Morel and Pochais\textsuperscript{15} found that varour given off at a temperature
of 37°C, by some volatile oils had a feeble germicidal effect but that none except the most sensitive bacteria were killed and those only after several hours.

Anisi oil has also been recommended as a means of destroying bodylice 'Partur' used 1% ointment of anisi oil for scabies. The essential oils are extraordinary active insecticides and useful to protect the body against lice and other vermin.\textsuperscript{16}

It was found that in the treatment of infection of the upper respiratory tract and certain type of skin diseases the essential oils are highly effective. A number of essential oils have thus been employed therapeutically. Antibacterial vapours of the volatile substances such as thymol, menthol etc., have even been used for many years for inhalation therapy in respiratory disorders of bacterial origin.\textsuperscript{17} A number of perfume oils were studied against bacteria and fungi by Maruzzella and Theny\textsuperscript{18} and they found interesting results.

Though the cost of the volatile oils precludes their use for routine disinfectant purpose, yet for their antibacterial properties in the mouth and nose, they may be valued agents. Many of the constituents of the essential oil, already enumerated, and other alcohols, aldehydes, ketones, esters etc., are now manufactured synthetically for use in perfumery and in medicines.
Essential oils of lavender, rosemary, mint and camphor are particularly effective for the treatment of the diseases of the scalp owing to the various phenols and higher alcohols which act as antiseptics.\textsuperscript{19} The eucalyptus oil has been found active against \textit{E. Coli} and the same oil has also been studied on the acid fast bacteria of tuberculosis and leprosy.\textsuperscript{20}

It was found that sandalwood, rose, neroli, citronella, geranium, jasmin, anise, ylang-ylang and lemon, oils exhibit antifungal activity. Cadocchi and Meunier\textsuperscript{21} exposed typhoid bacilli to the action of the pure oil and then seeded the organisms on the Agar. It was found that to kill Salmonella, Ceylon cinnamon oil took 12 minutes, clove oil 25 minutes, wild thyme oil 35 minutes and oil of germanium 50 minutes. A number of plants have recently been found to contain antibacterial active substances.

Osborn\textsuperscript{22} conducted an enormous survey of higher plants in search of antimicrobial substances towards \textit{Staph. aureus} and \textit{E. Coli} as a result of detailed investigations of individual plant species by him and other workers, it was revealed that the antimicrobial substances were widely distributed among the higher plants and particularly among the angiosperms. The inhibiting and detrimental effects of the volatile oils make the useful as bacteriostatic and fungistatic agents. The oils
of eucalyptus, pine, myrrh, violet, angelica, and thyme in
the decreasing order of efficiency, have a therapeutic value in
the diseases of the respiratory organs.

The volatile oils of turpentine, pine, anise, lemon
and eucalyptus and the related drugs increase the volume output
of the respiratory tract fluid. Oil of anise has been found to
be the most effective expectorant of this group. The eucalyptus
oil has also an expectorant and a direct action on the secretory
cells of respiratory tract.

Catti and Cayula tested various oils against the
cultures Staph aureus, S. Pyogenes, P. glaucum and A. albus and
grouped them according to their therapeutic effectiveness. Oils
of clove, peppermint, camphor and cinnamon are highly active
against the above micro-organisms. Oils of eucalyptus and
lavender are reasonably active, rose oil moderately and lemon
grass oil is inactive. It was found that sandalwood oil was
effective against M. Pyogenes at the dilution of 1 : 64000.

ORGANIC MATTER AND ANTIMICROBIAL ACTIVITY :

The bactericidal potency of the various essential
oils reviewed above may not always show results of direct practical
significance. In practice, however, an essential oil is seldom
used in the pure state to act on bacterial growth. Practical
efficacy of the disinfectant must, therefore, include a suitable form of organic matter in the test medium because the organic matter provides a congenial atmosphere for the bacteria to thrive.

Different workers have taken a wide variety of organic matter - Kemwood and Hewlett investigated in the presence of faeces, and Winter and Glyth used milk. Chick and Martin used 5% heat sterilised faeces. Carrot used 5% suspension of yeast instead of faeces as a standard form of organic matter.

WORK DONE:

It was thought worthwhile to proceed with microbiological studies with the following oils:
Zanthoxylum alatum, Kaempferia galanga, Pavonia odorata, Xanthium strumarium, Ophiophriza mungos, Andrographis perangansusa, Justicia procumbans and Anetium sowa, which have not so far been investigated for the purpose.

A comparative study of antimicrobial properties of the oils was carried out on both pathogenic and non-pathogenic bacteria and fungi at a different concentration. Bacteria which were taken up for the investigation together with more important diseases caused by them are listed below.
**B. anthracis:**

The cause of anthrax, an acute specific disease of cattle, sheep and swine, sometimes occurring in workers handling wood, and hides of animals. Two forms occur in man - cutaneous (malignant pustule) and internal anthrax. The burruncle ulcerates and discharges a seropurulent exudate and followed by septicemia. The internal or pulmonary type characterised by a pneumonia.

**E. Coli:**

Occasionally pathogenic to man, producing enteritis, peritonitis, cystitis, normally inhabitant of the intestine of man. In certain cases produces septicemia, peritonitis, inflammation of the liver and gall-bladder, cystitis, and meningitis.

**M. pertussis:**

The organism is commonly present in the normal nose and throat, causes scarlet fever, measles, whooping cough etc.

**S. aureus:** Found on skin and mucous membranes. The causative organism of boils, furuncles, abscesses and suppuration in wounds.

**S. aureusinae:** Same as S. aureus.

**S. leutea:** It is non-pathogenic one.
The more important fungi and the nature of the skin diseases caused by them are given below which were taken for present antifungal activity.31

**Dermatomycoses**: Refers to certain fungus infections of the skin caused by members of well defined group of fungi.

**A. niger and A. terreus**: They are the most common and trouble some fungus, some of which are pathogenic and can produce lesion in the tissues of man and animals. They are characterised by the presence of inflammatory granulomatous lesions in the skin, external ear, nasal sinuses, orbits of eyes, bronchi or lungs.

**C. tropicalis and C. albicans**: Ringworm of the feet is fungus infection caused by the various species of candida. The eruption most often is limited to the heels, soles and side of the feet.

**T. mentagrophytes**: Erosion Marginatum is a fungal infection caused by the various species of *Trichophyton*. The affected nails are discoloured and lustre-less, brittle and grooved as a result of this infection. The fungal infection of the beard area and face, neck caused by the various species of this group.

**M. canis**: Ringworm of the scalp is also a fungus infection of hair caused by species of *Microsporum* and *Trichophyton*. 
EXPERIMENTAL

AGAR DIFFUSION METHOD: (Cylinder method):

The above method was followed to study the antifungal and antibacterial properties of the essential oils. The experimental conditions were same throughout the investigations. Ethylene glycol was used as a solvent for the essential oils studied.

GLASSWARES:

Petri dishes, conical flasks, micro pipettes, test tubes, - all were cleaned well and dried, duly wrapped and sterilized in hot air oven at 150°C, for one hour.

MICROORGANISMS:

Twentyfour hours and fortyeight hours old cultures of bacteria and fungi respectively were used for testing of antibacterial and antifungal studies of the essential oils.

MEDIUM:

Oxoid nutrient broth, and nutrient agar were used for antibacterial activity and Mg broth and Mg agar medium were employed for the study of antifungal activities.

PREPARATION OF BROTH MEDIA FOR BACTERIA:
Lab-Lemco Beef extract 1 g.
Yeast extract (oxoid 20) 2 g.
Peptone (oxoid 57) 5 g.
Sodium chloride 5 g.
Distilled water 1 litre.
pH 7.4 (approx.)

The substances were weighed and dissolved in the freshly distilled water according to the above formula and volume was made up to 1 litre. 15 g./litre of agar-agar was added for nutrient agar media in addition to the above ingredients. Finally the pH was maintained 7.4. Both the broth and the media were transferred to a suitable conical flask, plugged and sterilized in autoclave at 15 lbs./pressure for 25 minutes.

PREPARATION OF M2 MEDIA FOR FUNGI:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerine</td>
<td>1.0%</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.5%</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0%</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>1.0%</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.01%</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>0.005%</td>
</tr>
<tr>
<td>pH</td>
<td>7.00</td>
</tr>
</tbody>
</table>
For N₂ agar media 2% (v/v) agar-agar was added in N₂ broth. pH was adjusted to 7.00. This was also sterilized by the same method. Glucose was added just before the sterilization.

SUBCULTURING AND PREPARATION OF SUSPENSION OF BACTERIA:

In a set of six test tubes about 2 ml. of nutrient broth per test tube was transferred. They were plugged, put in a beaker and sterilized by autoclaving. 5 ml. of melted nutrient agar was poured in each test tube of another set of six test tubes, plugged and autoclaved for sterilization and then cooled. The agar slants were used for subculturing bacteria. The time for the growth was 72 hours. The spores were suspended in 0.5% (v/v) Tween 80 solution in water.

ANTIBACTERIAL ACTIVITY METHOD:

Culture of each of the bacteria in nutrient broth was seeded in various petridishes with the help of micropipette in nearly 0.2 ml. suspension. Melted nutrient agar (20 ml.) was poured in each of the petri dishes, culture and media were mixed well and set aside for solidification. On solidifying four cylinders were placed in each agar plate of an external diameter 7 mm. Essential oils (in varying concentrations) in ethylene glycol were poured in respective cylinder in 0.05 ml. quantities. Each petri dish was labelled properly and incubated in an
electrically operated incubator, adjusted at 37°C for 24 hours. After 24 hours the zone of inhibition produced, was measured with the help of a divider and scale against a dark black ground. The results obtained with this concentration were promising.

**ANTIFUNGAL ACTIVITY METHOD**

The spores suspension of fungi (prepared as per method of subculturing of bacteria) was poured 1 ml. quantity in each petri dish. Melted M2 agar media (20 ml.) was used. The cylinders were placed in the usual manner and various solutions of essential oils (in varying concentrations) were poured in cylinders (0.5 ml. per cylinder). All the petri dishes were incubated at room temperature for nearly 3 days. Zone of inhibition produced was measured and the results are recorded in tables (I to VIII).
### TABLE I

**STUDY OF THE ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OILS.**

Concentration 1 : 100 dilution of zones of inhibition in mm.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Micro organisms</th>
<th>Ethyl Acetate</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>B. anthrax</td>
<td>Nil</td>
<td>26</td>
<td>Nil</td>
<td>21</td>
<td>19</td>
<td>16.5</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>S. aeruginosa</td>
<td>Nil</td>
<td>18</td>
<td>Nil</td>
<td>10.5</td>
<td>17</td>
<td>16</td>
<td>19</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>E. Coli</td>
<td>Nil</td>
<td>17</td>
<td>Nil</td>
<td>11</td>
<td>15</td>
<td>20</td>
<td>28</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>E. Pertusias</td>
<td>Nil</td>
<td>24</td>
<td>Nil</td>
<td>19</td>
<td>14</td>
<td>17</td>
<td>30</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>S. aureus</td>
<td>Nil</td>
<td>24</td>
<td>Nil</td>
<td>21</td>
<td>19</td>
<td>14</td>
<td>36</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>S. leutes</td>
<td>Nil</td>
<td>24</td>
<td>Nil</td>
<td>13</td>
<td>24</td>
<td>20</td>
<td>31</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

A = Zanthoxylum alatum.
B = Kaempferia galanga.
C = Pavonia odorata.
D = Xanthium strumarium.
E = Mungos.
F = Anisogon iwaransusa.
G = Justicia procumbens.
H = Anethum sowa.
ANTIBACTERIAL ACTIVITY
Andropogon lwarensus 1:100
Against - S. leutea

ANTIBACTERIAL ACTIVITY
Xanthium strumarium 1:100
Against - S. aureus

ANTIBACTERIAL ACTIVITY
Anethum sowa 1:100
Against - B. anthrax.
ANTIBACTERIAL ACTIVITY
Kaempferia galanga 1:250
Against - E. coli.
### TABLE II

**STUDY OF THE ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OILS.**

Concentration 1:250 diameter of zones of inhibition in mm.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Micro organisms</th>
<th>Ethylene glycol</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>B. anthrax</em></td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>18</td>
<td>NIL</td>
<td>11.5</td>
<td>13.5</td>
<td>9.5</td>
<td>NIL</td>
</tr>
<tr>
<td>2.</td>
<td><em>S. aeruginosa</em></td>
<td>NIL</td>
<td>NIL</td>
<td>19</td>
<td>9.5</td>
<td>NIL</td>
<td>NIL</td>
<td>9.5</td>
<td>11</td>
<td>NIL</td>
</tr>
<tr>
<td>3.</td>
<td><em>E. Coli</em></td>
<td>NIL</td>
<td>NIL</td>
<td>11.5</td>
<td>NIL</td>
<td>NIL</td>
<td>10</td>
<td>NIL</td>
<td>20</td>
<td>18.5</td>
</tr>
<tr>
<td>4.</td>
<td><em>H. Pertuis</em></td>
<td>NIL</td>
<td>NIL</td>
<td>14.5</td>
<td>NIL</td>
<td>9.5</td>
<td>11</td>
<td>NIL</td>
<td>22</td>
<td>9.4</td>
</tr>
<tr>
<td>5.</td>
<td><em>S. aureus</em></td>
<td>NIL</td>
<td>NIL</td>
<td>10.5</td>
<td>14</td>
<td>13</td>
<td>14</td>
<td>11</td>
<td>26</td>
<td>26.5</td>
</tr>
<tr>
<td>6.</td>
<td><em>S. leutea</em></td>
<td>NIL</td>
<td>NIL</td>
<td>9.5</td>
<td>NIL</td>
<td>NIL</td>
<td>18</td>
<td>13.5</td>
<td>24</td>
<td>NIL</td>
</tr>
</tbody>
</table>

A = Zanthoxylum alatum.
B = Kaempferia galanga.
C = Pavonia odorata.
D = Xanthium strumarium.

E = Ophiophriza mungo.
F = Andropogon iwaracusa.
G = Justicia procumbens.
H = Anethum cornum.
ANTIBACTERIAL ACTIVITY
*Justicia procumbens* 1:500
Against *S. aureus*

ANTIBACTERIAL ACTIVITY
of the essential oils
(1:1000)
no zone of inhibition
### TABLE III

**STUDY OF THE ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OILS.**

Concentration 1 : 500 diameter of zones of inhibition in mm.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Micro organisms</th>
<th>Ethylene glycol</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>B. anthrax</em></td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>9.5</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
</tr>
<tr>
<td>2.</td>
<td><em>S. aeruginosa</em></td>
<td>NIL</td>
<td>NIL</td>
<td>9.5</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
</tr>
<tr>
<td>3.</td>
<td><em>E. Coli</em></td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>11</td>
<td>9.3</td>
</tr>
<tr>
<td>4.</td>
<td><em>H. Pertusis</em></td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>14</td>
<td>NIL</td>
</tr>
<tr>
<td>5.</td>
<td><em>S. aureus</em></td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>16</td>
<td>17.2</td>
</tr>
<tr>
<td>6.</td>
<td><em>S. leutea</em></td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>9.1</td>
<td>NIL</td>
<td>13</td>
<td>NIL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A</th>
<th><em>Zanthoxylum alatum.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td><em>Kaempferia galanga.</em></td>
</tr>
<tr>
<td>C</td>
<td><em>Peyonia odorata.</em></td>
</tr>
<tr>
<td>D</td>
<td><em>Xanthium strumarium.</em></td>
</tr>
<tr>
<td></td>
<td><strong>E</strong> = <em>Ophiopogon mungos.</em></td>
</tr>
<tr>
<td></td>
<td><strong>F</strong> = <em>Andropogon iwarancusa.</em></td>
</tr>
<tr>
<td></td>
<td><strong>G</strong> = <em>Justicia procumbans.</em></td>
</tr>
<tr>
<td></td>
<td><strong>H</strong> = <em>Anethum sowa.</em></td>
</tr>
</tbody>
</table>
**TABLE IV**

**STUDY OF THE ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OILS**

Concentration 1 x 1000 diameter of zones of inhibition in mm.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Micro organisms</th>
<th>Ethylene glycol</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>B. anthrax</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
</tr>
<tr>
<td>2.</td>
<td>S. aeruginosa</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
</tr>
<tr>
<td>3.</td>
<td>E. Coli</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
</tr>
<tr>
<td>4.</td>
<td>H. Pertusis</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
</tr>
<tr>
<td>5.</td>
<td>S. aureus</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>11.0</td>
<td>11.5</td>
</tr>
<tr>
<td>6.</td>
<td>S. leutea</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
</tr>
</tbody>
</table>

A = Zanthoxylum alatum.
B = Kaempferia galanga.
C = Pavonia odorata.
D = Xanthium strumarium.
E = Ophiorrhiza mungos.
F = Andropogon iwarancusa.
G = Justicia procumbans.
H = Anethum sowa.
ANTIFUNGAL ACTIVITY
Kaempferia galanga 1:100
Against - C. albicans

ANTIFUNGAL ACTIVITY
Justicia procumbens 1:100
Against - T. mentagrophytes

ANTIFUNGAL ACTIVITY
Anethum scho 1:100
Against - A. tereus
TABLE V

STUDY OF THE ANTIFUNGAL ACTIVITY OF THE ESSENTIAL OILS.
Concentration 1 : 100 diameter of zones of inhibition in mm.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Micro organism</th>
<th>Ethylene glycol</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A. niger</td>
<td>Nil</td>
<td>Nil</td>
<td>19</td>
<td>31</td>
<td>28</td>
<td>41</td>
<td>22</td>
<td>31</td>
<td>17</td>
</tr>
<tr>
<td>2.</td>
<td>A. tereus</td>
<td>Nil</td>
<td>Nil</td>
<td>21</td>
<td>38</td>
<td>29</td>
<td>37</td>
<td>28</td>
<td>29</td>
<td>19</td>
</tr>
<tr>
<td>3.</td>
<td>M. canis</td>
<td>Nil</td>
<td>17</td>
<td>19</td>
<td>29</td>
<td>13</td>
<td>19</td>
<td>20</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>4.</td>
<td>Candida tropicalis</td>
<td>Nil</td>
<td>13</td>
<td>19</td>
<td>27</td>
<td>24</td>
<td>31</td>
<td>26</td>
<td>38</td>
<td>31</td>
</tr>
<tr>
<td>5.</td>
<td>Trichophton mentagrphteae</td>
<td>Nil</td>
<td>19</td>
<td>24</td>
<td>18</td>
<td>23</td>
<td>32</td>
<td>19</td>
<td>41</td>
<td>17</td>
</tr>
<tr>
<td>6.</td>
<td>Candida albicans</td>
<td>Nil</td>
<td>19</td>
<td>26</td>
<td>31</td>
<td>29</td>
<td>26</td>
<td>30</td>
<td>31</td>
<td>28</td>
</tr>
</tbody>
</table>

A = Zanthoxylum alatum.
B = Kaempferia galanga.
C = Pavonia odorata.
D = Xanthium strumarium.
E = Ophiorrhiza mungos.
F = Andropogon iwarancusa.
G = Justicia procumbans.
H = Anethum sowa.
ANTIFUNGAL ACTIVITY
Kamptfera galanga 1:250
Against - T. mentagrophytes.

ANTIFUNGAL ACTIVITY
Xanthium strumarium 1:250
Against - A. niger

ANTIFUNGAL ACTIVITY
Anethum sowa 1:250
Against - C. tropicalis
### TABLE VI

**STUDY OF THE ANTIFUNGAL ACTIVITY OF THE ESSENTIAL OILS**

Concentration 1: 250 diameter of zones of inhibition in mm.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Micro organism</th>
<th>Ethylene glycol</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A. niger</td>
<td>Nil</td>
<td>Nil</td>
<td>15</td>
<td>26</td>
<td>20</td>
<td>29</td>
<td>19</td>
<td>26</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>A. tereus</td>
<td>Nil</td>
<td>Nil</td>
<td>17</td>
<td>26.8</td>
<td>20.5</td>
<td>24</td>
<td>22</td>
<td>23.5</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>M. canis</td>
<td>Nil</td>
<td>11.5</td>
<td>14</td>
<td>21</td>
<td>9.5</td>
<td>13</td>
<td>14</td>
<td>11</td>
<td>9.5</td>
</tr>
<tr>
<td>4</td>
<td>Candida tropicalis</td>
<td>Nil</td>
<td>9.0</td>
<td>12.5</td>
<td>19</td>
<td>16.5</td>
<td>21</td>
<td>21</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>Trichophoton menagrophtea</td>
<td>Nil</td>
<td>14</td>
<td>18.5</td>
<td>11</td>
<td>16</td>
<td>20</td>
<td>17</td>
<td>27</td>
<td>11.5</td>
</tr>
<tr>
<td>6</td>
<td>Candida albicans</td>
<td>Nil</td>
<td>13.5</td>
<td>19.5</td>
<td>21</td>
<td>26</td>
<td>19</td>
<td>24</td>
<td>28</td>
<td>20</td>
</tr>
</tbody>
</table>

A = Zanthoxylum alatum.
B = Kaempferia galanga.
C = Pavonia odorata.
D = Xanthium strumarium.
E = Ophiopogon mungos.
F = Andropogon iararancusa.
G = Justicia procumbans.
H = Anethum sowa.
ANTIFUNGAL ACTIVITY
Andropogon-ivarancusa 1:500
Against - A. tereus
TABLE VII

STUDY OF THE ANTIFUNGAL ACTIVITY OF THE ESSENTIAL OILS.

Concentration 1 : 500 diameter of zones of inhibition in mm.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Micro organism</th>
<th>Ethylene glycol</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A. niger</td>
<td>NIL</td>
<td>NIL</td>
<td>9.5</td>
<td>13.5</td>
<td>11</td>
<td>17</td>
<td>11</td>
<td>19</td>
<td>NIL</td>
</tr>
<tr>
<td>2.</td>
<td>A. tereus</td>
<td>NIL</td>
<td>NIL</td>
<td>11</td>
<td>16</td>
<td>9</td>
<td>13</td>
<td>15.5</td>
<td>14.5</td>
<td>NIL</td>
</tr>
<tr>
<td>3.</td>
<td>M. canis</td>
<td>NIL</td>
<td>NIL</td>
<td>9</td>
<td>11</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
</tr>
<tr>
<td>4.</td>
<td>Candida tropicalis</td>
<td>NIL</td>
<td>NIL</td>
<td>9.5</td>
<td>9.5</td>
<td>NIL</td>
<td>14.5</td>
<td>16</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Trichophpton mentagrophytes</td>
<td>NIL</td>
<td>NIL</td>
<td>10</td>
<td>NIL</td>
<td>105</td>
<td>11</td>
<td>11</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>6.</td>
<td>Candida albicans</td>
<td>NIL</td>
<td>NIL</td>
<td>13</td>
<td>16.5</td>
<td>NIL</td>
<td>18</td>
<td>13.5</td>
<td>10.5</td>
<td></td>
</tr>
</tbody>
</table>

A = Zanthoxylum alatum.
B = Kaempferia galanga.
C = Pavonia odorata.
D = Xanthium strumarium.
E = Ophiithiza mungos.
F = Andropogon iwarangusa.
G = Justicia procumbana.
H = Anethium sowa.
TABLE VIII

STUDY OF THE ANTIFUNGAL ACTIVITY OF THE ESSENTIAL OILS

Concentration 1: 1000 diameter of zones of inhibition in mm.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Micro organism</th>
<th>Ethylene glycol</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A. niger</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>10</td>
<td>Nil</td>
</tr>
<tr>
<td>2.</td>
<td>A. tereus</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>9</td>
<td>Nil</td>
</tr>
<tr>
<td>5.</td>
<td>M. canis</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>4.</td>
<td>Candida tropicalis</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>11</td>
<td>Nil</td>
</tr>
<tr>
<td>5.</td>
<td>Trichophyton mentagrophytes</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>6.</td>
<td>Candida albicans</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Slight</td>
<td>Slight</td>
<td>Nil</td>
<td>9.5</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

A = Zanthoxylum alatum.  
B = Kaempferia galanga.  
C = Pavonia odorata.  
D = Xanthium strumarium.  
E = Ophiorrhiza mungos.  
F = Andropogon iwaransus.  
G = Justicia procumbens.  
H = Anethum sowa.
DISCUSSION

Antimicrobial activity of the essential oils have been compared by measuring the zone of inhibition.

While comparing the activity of the oils, it can be seen from the tabulated results that the essential oil of Justicia procumbens ('G' oil) exhibits the maximum microbial activity at 1 : 100 (zone of inhibition, 36 mm.). It is interesting to note that at the increasing dilution of 1 : 250, it has got good inhibitory activity on the growth of all organisms. Further at 1 : 500 dilution it shows reasonable inhibitory activity on all the organisms except against B. anthrax and S. aereauginosa. It is significant to note that even at a dilution of 1 : 1000 it has got some inhibitory activity against S. aureus (zone of inhibition 9.5 mm.).

The essential oil of Kaempferia galanga ('B' oil) was found to possess a little less antibacterial activity than the oil of Justicia procumbans ('G' oil). At a concentration of 1 : 100 it shows the maximum inhibitory activity against S. aereauginosa, (zone of inhibition 28 mm.) and has got a good overall inhibitory activity on the tested organisms at the above concentration. At 1 : 250 dilution the results were encouraging except against B. anthrax and at 1 : 500 concentration the only positive result obtained was against S. aereauginosa (zone of
inhibition 9.5 mm.), whereas no positive result was obtained at 1:1000 dilution.

Anethum sowa oil ('H' oil) can be placed third in order of antibacterial activity on the tested organisms. At 1:100 concentration the maximum inhibitory activity was found against S. aureus. At 1:250 dilution the activity of the oil was quite satisfactory except against B. anthracis and S. aeruginosa. Even at 1:500 it showed a reasonable antibacterial activity on E. coli (zone of inhibition 9.5 mm.) and S. aureus (zone of inhibition 17 mm.). The only positive result obtained at 1:1000 dilution was against S. aureus (zone of inhibition 9.5 mm.), whereas the essential oils of Pavonia odorata ('C' oil), Xanthium strumarium ('D' oil), Ophiopogon mungos ('E' oil) and Andropogon iveranusa ('F' oil) possess moderate antibacterial activity against the tested organisms, but the essential oil of Zanthoxylum alatum practically lacks the antibacterial effect.

Regarding the antifungal properties, it is interesting to note that Justicia procumbans ('G' oil) tops the list of the oils tested. Maximum activity was found against C. albicans (zone of inhibition 41 mm. at 1:100 concentration). It is to be noted that Justicia procumbans also showed the highest antibacterial activity among the oil tested. It gave positive results at 1:250 dilution and even at 1:500 except
against M. canis. Further this is the only oil which showed reasonable activity at 1:1000 dilution, whereas the other oils showed negative results at this concentration.

The next important oil for antifungal activity was found to be that of Pavonia odorata. It showed a good inhibitory activity. The maximum activity was found against A. tereus ("A" oil, zone of inhibition 38 mm.). A good antifungal activity was marked at 1:250 and 1:500 dilution but no activity was observed at 1:1000 dilution.

Almost similar antifungal activity was marked for Kaempferia galanga ("B" oil) and Anethum sowa ("H" oil) oils. Both of these gave negative results at 1:1000 dilution. At 1:250 dilution, good and at 1:500 dilution, moderate results were observed. The remaining oils of Zanthoxylum alatum ("A" oil), Xanthium strumarium ("D" oil), Androcoron ferrarum ("F" oil) and Ophiiorhiza mungos ("E" oil) though have a good antifungal activity at 1:100 concentration, lose it practically to negligible at increasing dilution.

From the tabulated results it can be seen that the essential oils have a good inhibitory activity on both the bacteria as well as the fungi. From the present investigations it can safely be concluded that the oil of Justicia procumbans ("F" oil) is the most effective antimicrobial agent and possesses a remarkable inhibitory activity. Further it would be worthwhile
to investigate the active component/components which are responsible for the microbial activity of the essential oils. This work could not be undertaken by the present author due to paucity of the authentic samples.

Thus from this preliminary investigation it can be suggested that the oils of Anethum and Kaempferia and their preparations can be used against skin disorders resulting from various micro-organisms.
SUMMARY

Essential oils of Zanthoxylum alatum, Kaempferia galanga, Pavonia odorata, Xanthium strumarium, Ophiopogon munsroi, Andropogon iwaranoensis, Justicia procumbens and Anethum sowa have been studied for their antimicrobial activity against certain pathogenic as well as nonpathogenic bacteria and fungi using cup cylinder method. The bacteria included for the study were B.anthracis, S. aeruginosa, E.coli, H.Pertussis, S.aureus and S.leutea and fungi were A.niger, A.tereus, M.canis, C.tropicalis, T.mentagrophytes and C.albicans.

The investigations showed all the essential oils to have potent antimicrobial activity against all the tested organisms at concentrations 1:100 and 1:250, but the essential oils of Anethum sowa and Kaempferia galanga were found to retain their activity even at increasing dilutions. The antifungal investigations were carried out with some of the important fungi responsible for the cause of common skin diseases; the results can be useful medicinally and serve to ameliorate human sufferings.
REFERENCES


9. Myers, Amer. J. Pharmacy, 1931, 103, 324.


