SYNERGISTIC EFFECT OF AZADIRACHTA INDICA L., ALOE VERA L. AND ANTIBIOTICS AGAINST E.COLI BACTERIUM.

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Accepted Date: 12/04/2015; Published Date: 27/04/2015

Abstract: Some plants are known as medicinal because they contain active substances that cause certain reaction, from relenting to the cure of disease. Aloe vera Linn. and Azadirachta indica are most important and common medicinal plants. The synergistic effect from the combination of antibiotics with plant extracts against resistant bacteria leads to new ways for the treatment of infectious diseases. Synergistic effect of different parts of Aloe vera (Liliaceae) and Azadirachta indica (Meliaceae) were tested against pathogenic bacteria to evaluate the possibility of new pharmaceutical. In the present study the synergistic effect of antibiotics ampicillin and tetracycllin with methanolic leaf and stem extracts of Azadirachta indica and leaf extract of Aloe vera in combination were seen against bacteria E.coli. Many combinations were seen more effectiveness than expected individually. However many combinations did not exhibit any synergistic effect.

Keywords: Aloe vera, Azadirachta indica, Plant extract, E.coli, Antibacterial, Synergistic
INTRODUCTION

Some plants are known as medicinal because they contain active substances that cause certain reaction, from relenting to the cure of disease. *Aloe vera* Linn. and *Azadirachta indica* are most important and common medicinal plants. *Aloe vera* is a succulent from the Aloe family (400 different species) with its African continent. *Aloe vera* leaves contain the water supply for the plant to survive long periods of drought. When a leaf is cut, an orange-yellow sap drips from the open end. When the green skin of a leaf is removed a clear mucilaginous substances appears that contains fibres, water and the ingredient to retain the water in the leaf, this is called gel. *Aloe vera* gel consists of 99.3% water. The remaining 0.7% is made up of solid with glucose and mannose constituting for a large part (Agarry O.O, 2005 [1]). *Azadirachta indica* (Family- *Meliaceae*) commonly known as *neem*, is native of India and naturalized in most of tropical and subtropical countries are of great medicinal value and distributed widespread in the world. The chemical constituents contain many biologically active compounds that can be extracted from *neem* including alkaloids, triterpenoids, phenolic compounds, carotenoids steroids and ketone, biologically most active compound is azadirachtin, it is actually a mixture of seven isomeric compounds labelled as azadirachtin A-G and azadirachtin E is more effective (Hashmat I. et al, 2012 [6]).

Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new, antimicrobial compounds. *Aloe vera* (family- Liliaceae) is a stem less plant. *Aloe vera* juice is of great medicinal importance and traditionally used as anti-inflammatory agent and in cosmetic industry. *Azadirachta indica* is well known for its medicinal properties. Its leaves possess broad spectrum of activity against Gram +ve and Gram –ve bacteria including *Mycobacterium tuberculosis*, *Vibrio cholera*. Different antibiotics exercise their inhibitory activity on various pathogenic organisms either by killing them outright (microbicidal action) or by arresting the growth of the causal organism (microbistatic action). The impact of antibiotic on a pathogen is specific which differs from pathogen to pathogen and vice-versa. The increasing and indiscriminate use of antibiotics has led to the development of bacterial resistance to antibiotics. Some studies in recent years have suggested the use of combination of antibiotics, the synergistic effect of which often surpasses their individual inhibitory activity. The use of synergistic combinations in antimicrobial chemotherapy is often used commercially for the treatment of various infections.

The development and spread of drug resistance among different bacteria to currently available antibiotic is a world wide concern. One strategy employed to overcome these resistance mechanism is the use of combination therapy. This therapy can be used to expand the antimicrobial spectrum, to prevent the emergence of resistant mutants to minimize toxicity, thereby exhibiting antimicrobial activity greater than that were expected from each
antimicrobial drug individually. It was therefore thought imperative to use an antibiotic in combination with leaf extracts of plants, with a number of therapeutic attributes to it. The synergistic effect from the combination of antibiotics with plant extracts against resistant bacteria leads to new ways for the treatment of infectious diseases. Synergistic effect of different parts of A. vera and A. indica were tested against pathogenic bacteria to evaluate the possibility of new pharmaceutical.

In the present study the synergistic effect of antibiotics ampicillin and tetracyclin with methanolic leaf and stem extracts of Azadirachta indica and leaf extract of Aloe vera in combination were seen against bacteria E. coli.

Material and Methods

Plant material:

The plants used in this study are Azadirachta indica, Aloe vera and were collected locally from Dayalbagh dairy area.

Preparation of the crude extracts:

Neem stem bark, and leaf extract –

The stem bark and leaf were cut into smaller pieces and dried in the laboratory, then the samples were powdered. Fifty grammes of each air dry powdered samples were extracted by mixing the 50ml methanol thoroughly shaken, and left for 24 hours at room temperature. The extracts were then filtered and different concentrations (30 ml, 70ml, 90ml) were prepared for experiments.

Aloe vera leaf extract –

To prepare crude extract of fresh A. vera whole leaves were washed with distilled water, chopped into small pieces, air-dried and ground into powder. The dried powder was extracted with 95% ethanol for one week. Then it was filtered through filter paper and different concentrations (30ml, 70ml, 90ml) were prepared for experiments.

Preparation of test organism:-

The normal agar were mixed well and poured on the sterile petri plates. The agar media on petri plates were allowed to set for few minutes. Normal agar plates were inoculated with
respective bacteria (*E. coli*), and then incubated at 37° C for overnight. Each time, a fresh bacterial culture was prepared.

**Method** –

**Antibacterial activity:**

Antibacterial activity was measured using paper disc diffusion method.

**Paper disc diffusion method, (Method of Saba Irshad et al, 2011 [18] was followed):**

The following steps were involved in paper disc diffusion method. The normal agar were mixed well and poured on the sterile petri plates. The agar media on petri plates were allowed to set harden for few minutes. Normal agar plates were inoculated with respective bacteria. The small autoclaved discs of whatmann filter paper were used. The test organism was spread on the petri plates by using sterilized glass spreader. During paper-disc diffusion method, the sterile discs were dipped in the different crude extracts of medicinal plants and antibiotic drugs with the help of sterilized forceps and placed on the Petri plates. Distilled water was used as a control to check the comparison of antibacterial activity with different crude extracts of medicinal plants. The petri plates were sealed with para film. Then, the petri plates were left at room temperature for 30 minute, to allow the diffusion of the test sample and then incubated at 37° C for overnight. The diameter of the zones of inhibition were measured in cm.

**Percentage inhibition was calculated as follows:**

\[
\text{Percentage inhibition} = \frac{\text{Treatment} - \text{control}}{\text{Treatment}} \times 100
\]

**Synergistic effect** –

Synergistic effect was evaluated using combinations of plant extracts of Neem (A) and *Aloe vera* (B), combination of antibiotics [ampicillin (1) and tetracyclin (2)], and antibiotics with plant extracts [Neem (A) and Aloe vera (B)], antibiotic (ampicillin) with plant extract of Neem (A), antibiotic (ampicillin) with plant extract of *Aloe vera* (B), antibiotic (tetracyclin) with plant extract of Neem (A), antibiotic (tetracyclin) with plant extract of *Aloe vera*., plant extracts [(A) and (B)] with tetracyclin, and plant extracts [(A) (B)] with ampicillin.

**RESULTS AND DISCUSSION**

- Results of Antibacterial activity of plant extracts and antibiotics against *E.coli* bacteria by using paper disc diffusion method are given in table no. 1 and figure no. 1.
Table no. -1 Antibacterial activity of plant extracts and antibiotics against *E.coli* bacteria by using paper disc diffusion method:-

<table>
<thead>
<tr>
<th>Samples</th>
<th>Concentrations (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percentage Inhibition</td>
</tr>
<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0</td>
</tr>
<tr>
<td>A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>46.81</td>
</tr>
</tbody>
</table>

Where-  A<sub>1</sub> = Neem leaf extract, A<sub>2</sub> = Neem bark extract, B = *Aloe vera* leaf extract, 1 = Ampicillin antibiotic, 2 = Tetracycllin antibiotic, control = distilled water.

Figure no. -1 Antibacterial activity of plant extracts and antibiotics against *E.coli* bacteria by using paper disc diffusion method

Amongst plant extracts used, maximum percent inhibition was observed in *Aloe vera* at 90 ml concentration followed by Neem at 90 ml concentration. The overall maximum percent inhibition value was obtained with ampicillin antibiotic at 90 ml concentration against *E. coli* bacteria. The minimum percent inhibition value showed *Aloe vera* leaf extract at 30 ml concentration. Neem leaf and bark extracts (at 30ml concentration) and ampicillin antibiotic (at 30ml and 70ml concentration) show zero percent inhibition against *E. coli* bacteria.
Evaluation of mean percentage inhibition of antibacterial effect of plant extracts (Neem and Aloe vera) and antibiotics (Ampicillin and Tetracyclin) against E. coli bacteria by paper disc diffusion method are presented in table no. 2 and figure no. 2.

**TABLE 2**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean Percentage inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁</td>
<td>42.66</td>
</tr>
<tr>
<td>A₂</td>
<td>11.82</td>
</tr>
<tr>
<td>B</td>
<td>43.22</td>
</tr>
<tr>
<td>1</td>
<td>27.10</td>
</tr>
<tr>
<td>2</td>
<td>50.28</td>
</tr>
</tbody>
</table>

**Figure no. 2** - Antibacterial effect by paper disc diffusion method:

![Figure 2](image_url)

Where - A₁ = Neem leaf extract

A₂ = Neem bark extract

B = Aloe vera leaf extract

₁ = Ampicillin antibiotic

₂ = Tetracyclin antibiotic

The maximum mean percent inhibition value amongst the plant extracts is seen in Aloe vera plant extract. However, Tetracyclin antibiotic shows maximum mean percent inhibition and minimum mean percent inhibition value was observed in Neem bark extract against E. coli bacteria.

- **Synergistic activity of plant extracts and antibiotic were also evaluated and the results are given in table no.3 and figure no.3.**
Table no. 3: Antibacterial activity of plant extracts and antibiotics in synergism against *E.coli* bacteria by using paper disc diffusion method:-

<table>
<thead>
<tr>
<th>Combinations</th>
<th>Percentage Inhibition (%)</th>
<th>Concentrations (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>A₁ + B</td>
<td>0</td>
<td>47.61</td>
</tr>
<tr>
<td>A₂ + B</td>
<td>37.94</td>
<td>0</td>
</tr>
<tr>
<td>1 + 2</td>
<td>25.42</td>
<td>29.41</td>
</tr>
</tbody>
</table>

Where - A₁ + B = Neem leaf extract + *Aloe vera* leaf extract

A₂ + B = Neem bark extract + *Aloe vera* leaf extract

1 + 2 = Ampicillin antibiotic + Tetracyclin antibiotic

- Figure no. 3: Antibacterial activity of plant extracts and antibiotics against *E.coli* bacteria by using paper disc diffusion method.

Where - A₁ + B = Neem leaf extract + *Aloe vera* leaf extract

A₂ + B = Neem bark extract + *Aloe vera* leaf extract

1 + 2 = Ampicillin antibiotic + Tetracyclin antibiotic
control = distilled water

The maximum percent inhibition value is seen in combination $A_2 + B$ i.e. Neem bark extract + Aloe vera leaf extract at 90 ml concentration against *E. coli* bacteria and minimum percent inhibition value was observed in combination $A_1 + B$ (Neem leaf extract + Aloe vera leaf extract) at 90 ml concentration against *E. coli* bacteria. Individual antibiotic were more effective. They showed negative synergism when used in combination.

- Mean percentage inhibition evaluated in table no. 4 and figure no. 4.

**Table no. 4-** Synergistic effect of plant extracts (Neem and Aloe vera) and antibiotics (Ampicillin and tetracyclin) against *E. coli* bacteria by using paper disc diffusion method:

<table>
<thead>
<tr>
<th>Combination</th>
<th>Mean percentage inhibition</th>
<th>Synergistic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_1 + B$</td>
<td>17.15</td>
<td>–</td>
</tr>
<tr>
<td>$A_2 + B$</td>
<td>31.80</td>
<td>–</td>
</tr>
<tr>
<td>$A_1 + 2$</td>
<td>30.85</td>
<td>–</td>
</tr>
</tbody>
</table>

- **Figure no.4** - Synergistic effect of plant extracts and antibiotics against *E. coli* bacteria by using paper disc diffusion method:

Where: – = no synergistic effect.

- $A_1$ = Neem leaf extract
- $A_2$ = Neem bark extract
- $B$ = Aloe vera leaf extract
- $1$ = Ampicillin antibiotic
- $2$ = Tetracyclin antibiotic
The maximum mean percent inhibition value is observed in $A_2 + B$ (Neem bark extract + Aloe vera leaf extract) against E. coli bacteria and minimum mean percent inhibition value is seen in $A_1 + B$ (Neem leaf extract + Aloe vera leaf extract) against E. coli bacteria. As such no positive synergistic effect was observed.

- Observation for synergistic effect of different combinations of plant extracts and antibiotics against E.coli bacteria by using paper disc diffusion method.

Mean percentage inhibition evaluated in table no. 5 and figure no. 5.

- Table no. 5 :- Synergistic effect of plant extracts and antibiotics against E.coli bacteria by using paper disc diffusion method.

<table>
<thead>
<tr>
<th>combinations</th>
<th>Mean percentage inhibition</th>
<th>Synergistic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 +A$_1$</td>
<td>47.27</td>
<td>+</td>
</tr>
<tr>
<td>1 + A$_2$</td>
<td>23.62</td>
<td>−</td>
</tr>
<tr>
<td>1 + B</td>
<td>45.44</td>
<td>+</td>
</tr>
<tr>
<td>2 + A$_1$</td>
<td>55.89</td>
<td>+</td>
</tr>
<tr>
<td>2 + A$_2$</td>
<td>71.15</td>
<td>+</td>
</tr>
<tr>
<td>2 + B</td>
<td>58.36</td>
<td>+</td>
</tr>
<tr>
<td>A$_1$ +B + 1</td>
<td>46.24</td>
<td>+</td>
</tr>
<tr>
<td>A$_2$ + B + 1</td>
<td>33.08</td>
<td>−</td>
</tr>
<tr>
<td>A$_1$ +B + 2</td>
<td>45.12</td>
<td>−</td>
</tr>
<tr>
<td>A$_2$ + B + 2</td>
<td>47.70</td>
<td>−</td>
</tr>
<tr>
<td>A$_1$ +B + 1 + 2</td>
<td>46.78</td>
<td>−</td>
</tr>
<tr>
<td>A$_2$ + B + 1 + 2</td>
<td>52.44</td>
<td>+</td>
</tr>
</tbody>
</table>

Figure no. 5 :- Synergistic effect of plant extracts and antibiotics against E.coli bacteria by using paper disc diffusion method.
Where-  $A_1 = $ Neem leaf extract , $A_2 = $ Neem bark extract, $B = $ Aloe vera leaf extract

$1 = $ Ampicillin antibiotic,  $2 = $ Tetracyclin antibiotic, $- = $ no synergistic effect, $+ = $ synergistic effect present.

The maximum mean percent inhibition value was observed in  $2 + A_2$ (combination of tetracyclin antibiotic and Neem bark extract) against $E..coli$ bacteria. Minimum mean percent inhibition value was observed in $1 + A_2$ (combination of Neem bark extract and ampicillin antibiotic) against $E.coli$ bacteria.

The positive synergistic effect was observed in $1 + A_1$, $1 + B$, $2 + A_1$, $2 + A_2$, $2 + B$, $A_1 + B + 1$, $A_2 + B + 1 + 2$ combinations as compared to their individual extracts.

CONCLUSION

Over all, individual plant extracts (Neem and Aloe vera) were less effective as compared to their different combinations with antibiotic drugs (ampicillin and tetracyclin). As per our review this synergism was reported for the first time and was the significant finding of the present work.

The synergistic effect may be due to certain complex formation which becomes more effective in the inhibition of a particular species of microorganisms either by inhibiting the cell wall synthesis or by causing its lyses or death. Thus it is concluded that to control a particular disease in vitro experiments should be carried out with various antibiotics and their combination as well as antibiotics and plant extracts, so that a right combination may be administered to the patient for early and safe recovery from a specific ailment. All the
combinations do not produce synergistic effect and therefore a number of combinations are 
inhibition value was observed in $2 + A_2$ (combination of tetracyclin antibiotic and Neem bark 
each) against $E.\text{coli}$ bacteria (table no. 6) as compared to their individual effect. But 
minimum mean percent inhibition value was observed in $1 + A_2$ (combination of Neem bark 
extract and ampicillin antibiotic) against $E.\text{coli}$ bacteria (table no. 6) as compared to their 
individual effect (table no.2).

Amongst plant extracts used, maximum percent inhibition was observed in $Aloe \text{ vera}$ at 90 ml 
concentration followed by Neem at 90 ml concentration. The overall maximum percent 
inhibition value was obtained with ampicillin antibiotic at 90 ml concentration against $E. \text{coli}$
bacteria. The minimum percent inhibition value showed $Aloe \text{ vera}$ leaf extract at 30 ml 
concentration. Neem leaf and bark extracts (at 30ml concentration) and ampicillin antibiotic 
(at 30ml and 70ml concentration) show zero percent inhibition against $E. \text{coli}$ bacteria.

The maximum mean percent inhibition value amongst the plant extracts is seen in $Aloe \text{ vera}$ 
plant extract. However, tetracyclin antibiotic shows maximum mean percent inhibition and 
minimum mean percent inhibition value was observed in Neem bark extract against $E. \text{coli}$ 
bacteria.

The maximum percent inhibition value is seen in combination $A_2 + B$ i.e. Neem bark extract + 
$Aloe \text{ vera}$ leaf extract at 90 ml concentration against $E. \text{coli}$ bacteria and minimum percent 
inhibition value was observed in combination $A_1 + B$ (Neem leaf extract + $Aloe \text{ vera}$ leaf extract) 
at 90 ml concentration against $E. \text{coli}$ bacteria. Individual antibiotic were more effective. They 
showed negative synergism when used in combination. The maximum mean percent inhibition 
value is observed in $A_2 + B$ (Neem bark extract + $Aloe \text{ vera}$ leaf extract) against $E. \text{coli}$ 
bacteria and minimum mean percent inhibition value is seen in $A_1 + B$ (Neem leaf extract + $Aloe \text{ vera}$ 
leaf tetracyclin extract) against $E. \text{coli}$ bacteria. As such no positive synergistic effect was 
observed. The maximum percent inhibition value was seen in $2 + A_1$ (combination of tetracyclin 
antibiotic and Neem leaf extract) at 90 ml concentration against $E. \text{coli}$ bacteria. Minimum mean 
inhibition value was observed in $2 + A_2$ (combination of antibiotic and Neem bark extract) against $E.\text{coli}$ bacteria. The maximum mean percent inhibition value was observed in $2 + A_2$ (combination of Neem bark extract and 
ampicillin antibiotic) against $E.\text{coli}$ bacteria.

The positive synergistic effect was observed in $1 + A_1, 1 + B, 2 + A_1, 2 + A_2, 2 + B, A_1 + B + 1,$ 
$A_2 + B + 1 + 2$ combinations as compared to their individual extracts) at 90 ml concentration 
against $E. \text{coli}$ bacteria, followed by minimum percent inhibition value was observed $A_2 + B + 1$ 
(combination of Neem bark extract, $Aloe \text{ vera}$ leaf extract, and ampicillin antibiotic) at 30 ml 
concentration against $E. \text{coli}$ bacteria.
So, the tested positive synergistic combinations can be used commercially for the treatment of various infection.

ACKNOWLEDGMENT

My heartfelt thanks to director of Dayalbagh Education Institute and Head of the Department, Department of Botany, for providing necessary facilities for the work.

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