The efficacy of polyherbal formulation of Moringa oleifera, Viola odorata, Allium sativum against microbes- synergistic effect

Meenakshi P Singh¹, Jagadeesh Singh SD¹*, Rajesh R²

¹*Department of Pharmacognosy & Biotechnology, East Point Research Academy, Bangalore- 560049, Karnataka, INDIA
²Department of Biotechnology, Tumkur University, Tumkur- 572106, Karnataka, INDIA

ABSTRACT

The purpose of our present study is to test antimicrobial and antifungal activity of polyherbal formulations. The testing of the antimicrobial activity on polyherbal formulation was done against gram positive (Staphylococcus aureus, Bacillus subtilis) and gram negative (Salmonella typhi, Escherichia coli) bacteria. The testing of antifungal activity was done against Aspergillus niger and Candida albicans with different combinations of polyherbal formulation. The microorganism to be tested was grown in relevant nutritional media. The solvent used for the extraction of Moringa oleifera, Viola odorata, Allium sativum was methanol. These methanolic extracts of chosen plants were further used in specific proportions for the preparation of polyherbal formulation (PF-1, PF-2, and PF-3). The resultant compositions were further used to check the efficacy against selected experimental microorganisms. PF-3 showed the significant high zone of inhibition against all the experimental microorganisms as compared to the other two combinations PF-1 and PF-2. Thus, the result has revealed that the antimicrobial activity is due to the synergistic effect of secondary metabolites present in these selected plants.

Introduction

The plant originated antimicrobials have huge therapeutic potential and are very effective in treatment of diseases infectious in nature. In addition to the treatment offered, they also help in extenuating side effects which are caused by synthetic antimicrobials. These side effects caused can be reduced through natural crude drugs [1]. The effectiveness of antimicrobial activities decides more therapeutic development of drugs. The antimicrobials research has been gradually reported in traditional medicinal plants [2]; still there is lack of systematic screening done against various microbes on the majority of traditionally used Indian medicinal flora [3].

The present research focused on three plants which have been used in the traditional system of medicine. These plants have been used traditionally in treatment of various ailments including infectious diseases. The treatment of microbial infection with drugs that are available is found ineffective due to microbial latency and conflicting efficiency in persistent infection among lower immunity [4] patients.

Corresponding Author: Dr. Jagadeesh Singh SD, Department of Pharmacognosy & Biotechnology, East Point Research Academy Banglore, Karnataka, India. E-Mail: meenakshipathania@gmail.com
wide range of ailments. The diverse combination of different plants would be more effective than individual plants itself as these formulations have the ability to act by various mechanisms [10]. Many numbers of plants and their products have been mostly reported for their significant antimicrobial activities. There is always a need to discover new leads of antimicrobial agents with potent chemical structures and effective mechanisms of actions as new and infectious diseases keeps arising everyday. There is a lot research being done towards traditional medicines as there is always need for novel drugs with improved bacterial resistance against infections [11, 12].

**Materials and methods**

**Plant materials**

The raw plant samples of *Moringa oleifera* (whole plant), *Viola odorata* (aerial parts) and *Allium sativum* (bulbs) were procured from Bangalore, India. The collected plant samples were identified and then authenticated by NISCAIR. The fresh samples were washed with distilled water, shade dried and homogenized to coarse powder and stored in the airtight containers.

**Extraction and formulation ratio**

Fifty grams (50g) of powdered plant leaves were taken in 250 ml of 95% methanol for 8-9 hours in Soxhlet apparatus. The extract was subsequently filtered by Whatman No.1 filter paper and allowed to evaporate and concentrate in at 60 ºC by using rotary evaporator. The resultant extract was then dissolved in Dimethyl sulfoxide (DMSO) and stored in refrigerator. All these three plants were extracted by methanol and then these methanolic extracts were further used for making different compositions of polyherbal formulations as shown in table 1.

<table>
<thead>
<tr>
<th>Formulation Name</th>
<th>Moringa oleifera</th>
<th>Viola odorata</th>
<th>Allium sativum</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF-1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PF-2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PF-3</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

**Microorganisms**

The investigated microorganisms consists of gram negative like *Escherichia coli*, *Salmonella typhi*, gram positive like *Staphylococcus aureus*, *Bacillus subtilis*, fungal strains *Aspergillus niger* and *Candida albicans* were used for investigation. The bacterial strains were grown in Mueller-Hinton agar plates at 37°C whereas the fungal test organisms were grown in Sabouraud dextrose agar, at 28°C. The stock cultures were retained at 4°C.

**Antimicrobial susceptibility test**

**Determination of zone of inhibition method**

The antimicrobial assay was carried out by agar well diffusion method [13]. The petri plates were prepared by using Muller Hinton Agar, after solidification 100 µl of bacterial culture was seeded on the particular plates. The sterile borer was used for boring holes and the holes was filled with 50mg/ml, 100mg/ml and 200mg/ml concentrations of test samples PF-1, PF-2, PF-3. The plates were incubated at 37°C for 24 hours whereas the fungal test organisms were cultivated in Sabouraud dextrose agar and incubated at 28°C for 24 hours. DMSO (Dimethysulphoxide) was used as vehicle control and while Standard drugs, streptomycin (100µg/ml) and nystatin (100µg/ml) were used as positive control for antibacterial and antifungal assays. The antimicrobial activity was then subsequently estimated by calculating the diameters of inhibitory zones in mm. All experimental assays were performed in triplicates and their mean was calculated.

**Results**

The results obtained to determine the inhibitory effect of different compositions of extract in polyherbal formulation. *Moringa oleifera, Viola odorata, Allium sativum* are traditionally used medicinal plants and the current study highlights the synergistic antimicrobial effect of them on the chosen bacterial and fungal strains. In all the cases the inhibitory effect of tested extracts was compared with standard antibiotics. The antibacterial effect of PF-1, PF-2 and PF-3 on the test samples and zones of inhibition are tabulated (Table- 2). The polyherbal formulation PF-3 showed the highest zone of inhibition as compared to another two polyherbal formulations PF-1 and PF-2. PF-3 exhibited the inhibitory effect on all the test samples. *S. aureus* showed the significantly highest zone of inhibition of 18.8mm whereas *S. typhi* showed the least of 10.6mm and while *B. subtilis* and *E. coli* has shown moderate zones respectively.

The antifungal effect of PF-3 formulation was best among others PF-1 and PF-2 formulations. *A.niger* showed the highest inhibition zone of 12.5mm which was followed by *C. albicans* with the inhibitory zone of 7.6mm. The results are tabulated (Table-3). The results of the antimicrobial sensitivity test were estimated to be statistically significant (p<0.05).
**Table 2:** Showing the bacterial zone of inhibition for different formulations PF-1, PF-2, PF-3

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>Formulation Concentrations</th>
<th>Positive control</th>
<th>Vehicle control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PF-1 (mg/ml)</td>
<td>PF-2(mg/ml)</td>
<td>PF-3(mg/ml)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>5 ± 0</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>5 ± 0</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>E. coli</td>
<td>5 ± 0</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>S. typhi</td>
<td>5 ± 0</td>
<td>100</td>
<td>200</td>
</tr>
</tbody>
</table>

Values are given in mean± standard deviation (SD). Experiments were carried in triplets (where n=3)

**Abbreviations:** S. aureus - Staphylococcus aureus, B. subtilis - Bacillus subtilis, E. coli - Escherichia coli, S. typhi - Salmonella typhi, - = No activity

**Table 3:** Showing the fungal zone of inhibition for different formulations PF-1, PF-2, PF-3

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>Formulation Concentrations</th>
<th>Positive control</th>
<th>Vehicle control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PF-1 (mg/ml)</td>
<td>PF-2(mg/ml)</td>
<td>PF-3(mg/ml)</td>
</tr>
<tr>
<td>A. niger</td>
<td>50</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>C. albicans</td>
<td>50</td>
<td>100</td>
<td>200</td>
</tr>
</tbody>
</table>

Values are given in mean± standard deviation (SD). Experiments were carried in triplets (where n=3)

**Abbreviations:** S. aureus - Staphylococcus aureus, B. subtilis - Bacillus subtilis, E. coli - Escherichia coli, S. typhi - Salmonella typhi, - = No activity
Values are given in mean± standard deviation (SD). Experiments were carried in triplets (where n=3)

**Abbreviations:** A. niger- Aspergillus niger, C. albicans- Candida albicans, - = No activity

**Discussion**

There was various phytochemicals reportedly found in different parts of plants, these phytochemicals acts as antimicrobial agents against pathogens. Microbial infections are increasing everyday that’s why advance research is required for the treatment of these infections. Even various synthetic antibiotics are known to cure microbial infections but still we cannot avoid their side effects, therefore phytomedicines are playing major role. The plant extracts have the ability to fight against these infections with improved efficacy and no or less side effects [14].

*Moringa oleifera, Viola odorata, Allium sativum* are already reported to have antimicrobial activity, and were used to prepare polyherbal formulation [15, 16, 17]. The methanolic extracts were selected because of high polarity and more extraction capacity of methanol. Due to this methanol extract has more number of quality phytochemicals that shows antimicrobial activity. The different compositions PF-1, PF-2 and PF-3 were formulated with selected plant extracts and later used to evaluate the antimicrobial efficacy against selected microorganisms. PF-3 had shown high antimicrobial activity than PF-1 and PF-2. The 200mg/ml concentration of PF-3 showed significantly good result.

The polyherbal formulation has been an effective approach for resistant strains of microbes. It is a novel concept because of the combination of more plants provides synergistic effect against infections.

The current study helped us to determine the antimicrobial activity of different combinations of polyherbal formulation against tested bacteria and fungi. *Moringa oleifera, Viola odorata, Allium sativum* were well known from traditional medicine, this study has showed the synergistic antimicrobial effect of these polyherbal formulations. It’s to be observed that inhibitory effect is due to diverse secondary metabolites present in the polyherbal formulation. The result has described the most effective antimicrobial activity of PF-3 against the tested gram positive and negative bacteria —such as *Streptococcus aureus, Bacillus subtilis, Salmonella typhi* and *Escherichia coli* and fungal species such as *Candida albicans and Aspergillus niger*. Therefore, present study proves that the traditional medicines are also more efficient, safe, economical and competent for the treatment of human pathogenic microorganisms.

**Acknowledgement**

The authors are thankful to Mr. R Rajendran and his GreenChem staff for providing support and helping them in conducting this research work.

**Conflict of interest:** We declare that we have no conflict of interest.

**References**

14. Yadav M., Chatterji S., Yadav D.K., Mubayi A., Watal G., Improved antibacterial efficacy of a novel...
