Antimicrobial analysis of Triphala and comparison with its individual constituents

Darshna Mahajan 1*, Sapna Jain2

1Research Scholar, Dept. of Biotechnology, IPS Academy Indore, M.P. India
2Research Scholar, Dept. of Biotechnology, IPS Academy Indore, M.P. India

ARTICLE INFO:

Article history:
Received: 29 June 2015
Received in revised form: 20 July 2015
Accepted: 20 August 2015
Available online: 30 September 2015

Keywords:
Antibacterial activity,
Antifungal activity,
Cup-plate method,
Triphala

ABSTRACT
Triphala, a well known ayurvedic formulation is used against number of ailments since ancient times. It consists of Emblica officinalis, Terminalia chebula and Terminalia bellerica in equal proportion. Triphala as a whole and its three individual constituents show specific antimicrobial activity against certain bacteria and fungi. Antimicrobial activity of aqueous extract of triphala and its constituents was studied against P. aeruginosa, E. coli, B. subtilis, K. pneumoniae and S. aureus by cup-plate method. Triphala was found strongly bactericidal against P. aeruginosa with 1.8 cm of inhibitory zone. This was on account of T. chebula which showed 1.2 cm of inhibitory zone against the same pathogen, followed by E. coli and other two Gram positive bacteria. T. bellerica however showed maximum inhibitory activity against B. subtilis by showing 2.2 cm of inhibitory zone. It was confirmed that antimicrobial activity against Gram negative bacteria is due to T. chebula and E. officinalis while antimicrobial activity against Gram positive bacteria is on account of T. bellerica. Antifungal activity of triphala and its constituents was studied against two pathogenic fungi viz. Aspergillus niger and Candida albicans by the same method. Triphala was found more efficient against A. niger, this was on account of T. bellerica which showed nearly 2 cm zone of inhibition. T. bellerica and Triphala showed more than 2 cm inhibitory zone against C. albicans. Inhibitory zone of Emblica officinalis was observed to be of 1.5-2 cm against both the fungi, whereas T. chebula did not show inhibitory activity against C. albicans but showed nearly 1 cm of inhibitory zone against A. niger. This confirms that the antifungal activity of Triphala is primarily due to T. bellerica and E. officinalis.

Introduction
Ayurveda is an ancient system of medicine in India which is based on balancing the three basic elements vat (space Air), pitta (energy and liquid) and kapha (liquid and solid) together known as tridosha (three pillars of life). Authentic information on ayurveda has been compiled by ancient India medicine practitioners in forms such as Charak Samhita, Sushrut Samhita etc. Triphala is one of the important formulations of ayurveda, which plays important role in balancing tridosha. The three constituents of Triphala namely Emblica officinalis, Terminalia chebula and Terminalia bellerca have been reported to show antimicrobial activity against various fungi and Gram positive and Gram negative bacteria. On account of this property, Triphala is popularly being used since ancient times to treat beneficial biological activities in mammals which include antiviral, immunostimulant, antiallergic, hepatoprotective and anticarcinogenic activity[1-8]. The present paper elaborated the comparative account of antimicrobial activity of Triphala and its various components against five human pathogenic bacterial and two fungi. Gram positive bacterial species selected for the purpose of study were Bacillus subtilis and Staphylococcus aureus and Gram negative bacteria were Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumonia. Aspergillus niger and Candida albicans were the two pathogenic fungi selected for the present study[10].

Materials and methods
Raw material triphala and its constituents (Amla, Har, Bahera) were procured from local markets. The dried fruits were purchased from the markets. They were then dried and powder was prepared which was again dried in an oven to remove traces of moisture these powder were then used for extract preparation.

Preparation of Triphala
Triphala was prepared by mixing all of the three constituents powders in ratio of 1:1:1 For this all the three constituent...
powders were weighed separately and then mixed to get triphala powder.

**Calculation of % LOD (loss on drying)**
The % LOD of the above prepared samples was calculated using the method. Weigh a glass petriplate that has been previously dried at 105 ° C, transfer about 1.0gm of the sample in the petriplate, distribute as evenly as possible, cover it and accurately weigh the petriplate and the contents. Place the loaded petriplate in the oven, temperature of which is maintained at 105 ° C and remove the cover, leave it also in the oven. Dry the sample for 4 hours. Remove the petriplate from the oven, close the cover. When cooled to room temperature, weigh it along with it’s cover. Note the loss on drying and again place the petriplate along with it’s cover open in the oven for one hour. Note the loss on drying and if the difference between two consecutive readings is less than 1.0mg, report the value in terms of percentage loss. If the difference is more than 1.0mg continue heating, cooling and weighing till the desired difference in weight is obtained. The LOD is calculated by using following formula:

\[
\text{Loss on drying in \% w/w} = \frac{(A-B) \times 100}{A}
\]

Where A: weight of sample before drying
B: weight of sample after drying

### Table 1: Calculation of % LOD (loss on drying)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wt. plates before sample</th>
<th>Wt. of plates putting (A) sample</th>
<th>Wt. after 4 hrs after drying</th>
<th>Wt. loss</th>
<th>Wt after 1 hrs after drying (B)</th>
<th>% LOD = (A-B) x100 A</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMLA</td>
<td>88.40</td>
<td>89.44</td>
<td>89.40</td>
<td>0.04</td>
<td>89.33</td>
<td>0.122</td>
</tr>
<tr>
<td>HAR</td>
<td>89.98</td>
<td>91.01</td>
<td>90.91</td>
<td>0.10</td>
<td>90.91</td>
<td>0.109</td>
</tr>
<tr>
<td>BAHERA</td>
<td>91.82</td>
<td>92.83</td>
<td>92.77</td>
<td>0.06</td>
<td>92.72</td>
<td>0.118</td>
</tr>
<tr>
<td>TRIPHALA</td>
<td>93.48</td>
<td>94.5</td>
<td>94.45</td>
<td>0.05</td>
<td>94.39</td>
<td>0.116</td>
</tr>
</tbody>
</table>

**Preparation of aqueous extracts**
Aqueous extracts of triphala were prepared by hot maceration method. 5gm of all dried powders were taken separately in a pestle and mortar and macerated with 40ml of luke warm water. The volume was made upto 50ml. The contents were then kept in an oven at 80 ° C. The extracts were then filtered using coarse filter paper which was pre-weighed and dried after filtering and weighed again. The solvent was removed using Rotary evaporator till 20ml was left. The extracts were stored in an airtight glass bottles in refrigerator [9].

**Setting The pH of the prepared extracts**
The pH of all the above prepared extracts was found by immersing electrodes of the pH meter, that has been standardized using appropriate buffer solution at 25 ° C and the pH of all the samples was set to neutral at the end.

### Table 2: Determination of PH of the prepared extracts

<table>
<thead>
<tr>
<th>Sample</th>
<th>Normal PH</th>
<th>After setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMLA</td>
<td>4.43</td>
<td>7</td>
</tr>
<tr>
<td>HAR</td>
<td>3.95</td>
<td>7</td>
</tr>
<tr>
<td>BAHERA</td>
<td>4.81</td>
<td>7</td>
</tr>
<tr>
<td>TRIPHALA</td>
<td>5.91</td>
<td>7</td>
</tr>
</tbody>
</table>

**Calculation of active component**
The amount of active component of the above prepared extracts was calculated as below: For this the filter papers through which the extracts are to be passed are weighed. 5gm of the each of the sample was dissolved in 40ml distilled water by hot maceration method. These were then passed through the pre-weighed filter paper, the filter paper was then dried in an hot air oven and then weighed again with the dried material left on it. The active component in the filtered extract was calculated as shown in the table 3.

### Table 3: Calculation of amount of active component in each sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wt. of paper filtering (gm)</th>
<th>Wt. of paper drying (y)</th>
<th>Remaining (x-y)</th>
<th>Active component in 25ml (gm)</th>
<th>Active component in 1 ml (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMLA</td>
<td>1.2</td>
<td>3.67</td>
<td>1.33</td>
<td>53.2</td>
<td></td>
</tr>
<tr>
<td>HAR</td>
<td>1.17</td>
<td>3.45</td>
<td>1.55</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>BAHERA</td>
<td>1.09</td>
<td>3.60</td>
<td>1.40</td>
<td>56</td>
<td></td>
</tr>
</tbody>
</table>
Testing antimicrobial activity of Triphala by Cup-plate

Suspension was formed.

peptone, 1000ml distilled water; pH 6.5) and a uniform cultures were added to the Sabouraud broth (40g dextrose, 10g

Fungal inoculum

Antimicrobial activity was checked by agar gel diffusion method. The cultures were grown in nutrient broth and incubated at 37°C for 24h. After incubation period was finished the zone of inhibition was measured and recorded [10-12].

Results and Discussion

Antimicrobial activity of various constituents of Triphala and Triphala as a whole was observed as a zone of inhibition against individual test microorganism. Triphala and T.chebula showed highest antibacterial activity against P. aeruginosa by exhibiting 1.8 and 1.2 cm zone of inhibition respectively (Table.1 and Fig.1).Triphala as compared to its constituents was found to be most effective against E.coli by exhibiting 1.7 cm of zone of inhibition. T. chebula showed 1.4 cm of zone inhibition where as E. officinalis and T. bellerica showed 1.2 cm of zone of inhibition. Comparative account of bactericidal activity of three constituents of triphala against B. subtilis showed that maximum inhibitory zone of 2.2 cm was exhibited by T. chebula and 0.6 cm by E. officinalis. Whereas, 1.6 cm of inhibitory zone was observed as a synergistic effect of three triphala constituents against the same pathogen K. pneumonia, a Gram negative bacteria, exhibited 1.4 cm of inhibitory zone demonstrating a synergistic activity of the three constituents against the bacteria. E. officinalis showed 0.9 cm of zone of inhibition and T.bellerica exhibited 0.7 cm of inhibitory zone. On the other hand, T.chebula did not showed any inhibitory effect against K.pneumonia, P.aeruginosa, other Gram negative bacteria, showed 1.8 cm zone of inhibition of Triphala which was maximum among all the five tested bacteria. T.chebula showed 1.1 cm of inhibitory zone against the same pathogen and 0.8 cm of inhibitory zone was exhibited by E.officinalis and T. bellerica each. S.aureus was found to be most sensitive for T.bellerica by exhibiting 1.6 cm of inhibitory zone followed by T.chebula, Triphala and E.officinalis by showing 1.2, 1.0 and 0.6 cm of inhibition zones respectively (Table 4 and Fig.1.1).

Triphala showed 2.3 and 2.2 cm of inhibitory zone against A. niger and C. albicans respectively. However, against A. niger, E. officinalis exhibited 3.4 cm of inhibitory zone followed by T. bellerica and T.chebula which exhibited 1.6 and 1.2 cm of zones of inhibition respectively. On the other hand, T. bellerica showed 1.5 cm of inhibition zone against C. albicans and E. officinalis showed 1.2 cm of zone of inhibition against the same pathogen. However, no zone of inhibition was exhibited by T.chebula against the same pathogenic fungi. (Table 5 and Fig.1.2).

Table 4: Diameter (cm) of zones of inhibition obtained against five pathogenic bacterial species

<table>
<thead>
<tr>
<th>Name of the organism</th>
<th>Triphala</th>
<th>E. officinalis</th>
<th>T. chebula</th>
<th>T. bellerica</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>1.7</td>
<td>1.2</td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>1.8</td>
<td>0.8</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>1.4</td>
<td>0.9</td>
<td>Nil</td>
<td>0.7</td>
</tr>
<tr>
<td>S. aureus</td>
<td>1.6</td>
<td>0.6</td>
<td>1.2</td>
<td>1.6</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>1.6</td>
<td>0.6</td>
<td>1.4</td>
<td>2.2*</td>
</tr>
</tbody>
</table>

*Maximum inhibitory zone.
Table 5: Diameter (cm) of zones of inhibition against *A. niger* and *C. albicans* for *Triphala* and its constituents

<table>
<thead>
<tr>
<th>Name of the organism</th>
<th><em>Triphala</em></th>
<th><em>E. officinalis</em></th>
<th><em>T. chebula</em></th>
<th><em>T. bellerica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. niger</em></td>
<td>2.3</td>
<td>3.4*</td>
<td>1.2</td>
<td>1.6</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>2.2</td>
<td>1.2</td>
<td>0</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*Maximum inhibitory zone.*

Fig. 1.1: Comparative chart of zones of inhibition obtained against all the five pathogenic bacterial species

Fig. 2.2: Comparative chart of zones of inhibition obtained against *A. niger* and *C. albicans*
Effect of Triphala on *E. coli*  
Effect of Triphala on *B. subtilis*

Effect of Triphala on *K. pneumoniae*  
Effect of Triphala on *P. aeruginosa*

FIG. 1.1: ANTIMICROBIAL ACTIVITY OF TRIPHALA AGAINST BACTERIAL SPECIES

Effect of Triphala on *C. albicans*  
Effect of Triphala on *A. niger*

FIG 2.1: ANTIMICROBIAL ACTIVITY OF TRIPHALA AGAINST FUNGAL SPECIES
On account of the observation it was concluded that, antibacterial activity against Gram negative bacteria is due to T.chebula and E.officinalis, while antibacterial activity against Gram positive bacteria is on account of T.bellerica.

Acknowledgements

We thankful to our guide Dr. Sanjay Nagar (Head), and management of IPS Academy, Indore for providing us timely help regarding laboratory facilities and other resources. We further wish to thank Mr. Mangilal waskel for providing computational assistance.

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

References


Triphala showed maximum antimicrobial activity against fungi by exhibiting more than 2cm of inhibition zone as compared to bacteria.


Cite this article as: Darshna Mahajan, Sapna Jain, Antimicrobial analysis of Triphala and comparison with its individual constituents. Indian J. Pharm. Biol. Res.2015; 3(3):55-60.