Antimicrobial (Bacteria, Fungi) Activity of Andrographis Paniculata and Detection of Andrographolide through TLC & HPLC Technique

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ABSTRACT
Andrographis paniculata is an annual herbaceous plant belonging to acanthaceae family it widely distributed on Asian countries. It’s height about 30-110cm, its flowers having a rose purple spots on their petals. It is having a dark green stem about 0.3-1.0 m in height and 2-6 nm in diameter. Antifertility activity. These are the main pharmacological potential of Andrographis paniculata. These are the main pharmacological potential of Andrographis paniculata. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of neem and reemerging infectious disease. Another big concern is the development of resistance to the antibiotics in current clinical use. In addition the allopathic medicines are not curing all the human diseases for Eg; Jaundice, cancer, paralysis, diabetics, and skin diseases etc., which are not curable by allopathic medicines. Hence the perception of medicine turned around towards the herbal plants. There are five cultures were tested in this project, among that three of them is bacteria and the remaining two of that are fungi bacteria i) vibrio cholera ii) klebsiella pneumonia iii) salmonella typhi. Fungi Candida albicans, cryptococci. All of these cultures are purchases from Tamilnadu Agricultural University (TNAU) Coimbatore. From this current investigation it was observed that the methanolic, petroleum ether and dichloromethane extract of Andrographis paniculata inhibited the growth of human pathogens 90%, 0% and 40% respectively. The above findings recommend the further investigations of Andrographis paniculata to evaluate their potential for use as antimicrobials to plant pathogens. The developed HPLC method can be utilized for the quantitative determination of andrographolide in Andrographis paniculata herb samples, extracts and dosage forms. HPTLC method also can utilized for quantitative determination of Andrographis paniculata herb samples, extracts and dosage forms. The complexity of the chemical composition of herbal extracts, quality of the herbal extracts can only be assured by the use of validated analytical methods for identification and quantification of the active ingredients. The HPLC and HPTLC methods for the quantitative estimation of andrographolide were validated with regard to their specificity, precision, accuracy and linearity. At the same retention time the leaf extract of Andrographis paniculata having a high amount of Andrographolide compare than their standard.

1. Introduction

Andrographis paniculata is an annual herbaceous plant belonging to acanthaceae family it widely distributed on Asian countries. It’s height about 30-110cm, its flowers having a rose purple spots on their petals. It is having a dark green stem about 0.3-1.0 m in height and 2-6 nm in diameter. Antithrombogenic activity, Antimalarial activity, Anti-inflammatory activity, Antisnakevenom activity, respiratory system benefits, and antifertility activity. These are the main pharmacological potential of Andrographis paniculata. These are the main pharmacological potential of Andrographis paniculata. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of neem and reemerging infectious disease. Another big concern is the development of resistance to the antibiotics in current clinical use, In addition the allopathic medicines are not curing all the human diseases for Eg; Jaundice, cancer, paralysis, diabetics, and skin diseases etc., which are not curable by allopathic medicines. Hence the perception of medicine turned around towards the herbal plants.

Anti-microbial activity and detection of Andrographolide through HPLC and TLC techniques.

a) Enumerate the antibacterial activity of Andrographis paniculata
b) Enumerates the antifungal activity of Andrographis paniculata
c) Detection of andrographolide through HPLC & TLC techniques.

2. Materials and Methods
There are five cultures were tested in this project, among that three of them is bacteria and the remaining two of that are fungi bacteria i) vibrio cholera ii) klebsiella pneumonia iii) salmonella typhi. Fungi Candida albicans, cryptococci. All of these cultures are purchases from Tamilnadu agricultural university (TNAU) Coimbatore.

Plant Samples
The leaf samples were purchased from ABS botanical garden, karipatti, Salem. The leaf of the Andrographis

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Paniculata is collected for this work, these leaves were allowed to shade dry at room temperature (28\(^\circ\)C ± 20\(^\circ\)C) for 4-7 days. The dried samples were ground by using a mixer and the fine powder were kept in airtight containers and stored in room temperature in a dark place.

**Antimicrobial Screening:**

The petroleum Ether, Methanol, Dichloro methane extracts of the *Andrographis paniculata* were screened for antimicrobial activity by Disc Diffusion Method.

### 3. Results and Discussion

The efficacy of three different extracts of *Andrographis paniculata* revealed that methanol extracts were more potent than petroleum ether followed by dichloromethane extract of *Andrographis paniculata*. All human pathogens were highly susceptible to methanol extract with variable concentrations viz., 10ml, 20ml and 25ml/well. Methanolic extracts showed maximum activity against three bacterial and two fungal strains. The methanolic extracts show the antimicrobial activity against all tested organisms, because methanol is a high polar in nature so all the active molecules of *Andrographis paniculata* is moved from the leaf extract easily.

The petroleum ether did not show the antimicrobial activity so the active molecules do not move from the leaf extract. Antimicrobial activity against limited number of tested organism was reported with Dichloro methane extract of andrographis paniculata. From this current investigation it was observed that the methanolic, petroleum ether and dichloromethane extract of *Andrographis paniculata* inhibited the growth of human pathogens 90%, 0% and 40% respectively. The above findings recommend the further investigations of *Andrographis paniculata* to evaluate their potential for use as antimicrobials to plant pathogens.

**Discussion for TLC &HPLC:**

1. The complexity of the chemical composition of herbal extracts, quality of the herbal extracts can only be assured by the use of validated analytical methods for identification and quantification of the active ingredients. The HPLC and HPTLC methods for the quantitative estimation of andrographolide were validated with regard to their specificity, precision, accuracy and linearity.

**Table 1. Effect of the three extracts of Andrographis paniculata on Candida albicans by disc diffusion method.**

<table>
<thead>
<tr>
<th>Name of the extract</th>
<th>Zone of Inhibition (Dia)</th>
<th>Concentration</th>
<th>Test Sample</th>
<th>+ve (Chloramphenicol)</th>
<th>-ve (solvent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether extract</td>
<td></td>
<td>10ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20ml</td>
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<td></td>
<td></td>
<td>25ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methanol extract</td>
<td></td>
<td>10ml</td>
<td>1.5 Dia</td>
<td>2.5 Dia</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20ml</td>
<td>2.4 Dia</td>
<td>2.9 Dia</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td>25ml</td>
<td>3.0 Dia</td>
<td>3.4 Dia</td>
<td>-</td>
</tr>
<tr>
<td>Dichloro methane extract</td>
<td></td>
<td>10ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20ml</td>
<td>-</td>
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<td></td>
<td></td>
<td>25ml</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>
Table 2. Effect of the three extracts of Andrographis paniculata on Cryptococci by disc diffusion method

<table>
<thead>
<tr>
<th>Name of the extract</th>
<th>Concentration</th>
<th>Test Sample</th>
<th>+ ve (Chlorempenicol)</th>
<th>-ve (solvent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether extract</td>
<td>10ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>20ml</td>
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<tr>
<td></td>
<td>25ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>10ml</td>
<td>1.7 Dia</td>
<td>2.0 Dia</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20ml</td>
<td>2.0 Dia</td>
<td>2.5 Dia</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>25ml</td>
<td>2.6 Dia</td>
<td>3.0 Dia</td>
<td>-</td>
</tr>
<tr>
<td>Dichloro methane extract</td>
<td>10ml</td>
<td>1.4 Dia</td>
<td>1.0 Dia</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20ml</td>
<td>1.6 Dia</td>
<td>1.2 Dia</td>
<td>-</td>
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<tr>
<td></td>
<td>25ml</td>
<td>1.9 Dia</td>
<td>2.0 Dia</td>
<td>-</td>
</tr>
</tbody>
</table>

Detection of Andrographolide

Calculate the percentage from Peak areas in HPLC

HPLC and TLC

Compare than the other standard the 1g of test sample having a high amount of andrographolide it can identified at the same retention time.
4. Conclusion

From this current investigation it was observed that the methanolic, petroleum ether and dichloromethane extract of Andrographis paniculata inhibited the growth of human pathogens 90%, 0% and 40% respectively. The above findings recommend the further investigations of Andrographis paniculata to evaluate their potential for use as antimicrobials to plant pathogens.

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