EVALUATION OF ANTI-MICROBIAL ACTIVITY IN PICRORHIZA KURROA PLANT EXTRACT USING THIN-LAYER CHROMATOGRAPHY AND FTIR

Jannathul Firdous1*, Bharathi2, Noorzaid Muhamad1 and Fazlin zaini1

1Pre-clinical Department, Faculty of Medicine, Universiti Kuala Lumpur Royal College of Medicine Perak, No.3, Jalan Greentown, 30450, Ipoh, Perak, Malaysia.
2Shrimati Indira Gandhi College, Near Chatiram Bus stand, Tiruchirappalli, India.

Email: Jannathul.firdous@unikl.edu.my

Received on 28-07-2016 Accepted on 01-09-2016

Abstract

Medicinal plants are the alternative remedy for antibiotics in treating human diseases for centuries because they contain numerous active constituents of therapeutic importance. The medicinal value of plant extracts is due to their target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. In the present study, the antimicrobial activity of Picrorhiza kurroa extract was analysed and evaluated using thin layer chromatography and fourier transform infra-red spectroscopy. The phytochemical screening of the plant extract in previous studies done by us showed the presence of alkaloids, tannins, steroids, flavonoids, saponins, tannins and phenolics. The presence of various bioactive compounds justifies the use of the plant for various ailments by traditional practitioners. As a result, Picrorhiza kurroa extract possesses antimicrobial activity as the zone of inhibition was observed for both gram positive as well as gram negative bacterial strains. The organic compounds responsible for such activity was also evaluated using TLC and FTIR.

1. Introduction

Natural compounds isolated from plants are found to be a natural immediate sources for next to antibiotics. There is a growing importance and interest to use plants and spices for the eradication of microorganisms because of their increasing antibiotic resisting ability [1]. This natural plant products includes aromatic substances such as phenols and their oxygen-substituted derivatives like tannins. Compounds with alkaloids property serve as plant defense mechanisms by performing predatory action against microorganisms, insects and herbivores. Herbs and spices used by us to season food for favoring or coloring yield useful medicinal compounds [2]. In addition, public interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug resistance reactions and economic burden of the modern medicine [3].
Therefore, plant derived products can be expanded with sustainable, comparative and competitive advantage. These include cost effective. *Picrorhiza kurroa* is one among the medicinal herb, grows in the hilly regions of India and Nepal.

*Picrorhiza kurroa* contains apocynin, a powerful anti-inflammatory agent that reduces platelet aggregation. Traditional methods utilizing *Picrorhiza kurroa* used to treat liver and upper respiratory conditions in India and China.

In this study, organic compounds responsible for the anti-microbial activity in *Picrorhiza kurroa* were identified using Fourier transform infrared spectroscopy (FTIR) and those compounds were separated using thin layer chromatography. Further, antimicrobial activity was analyzed by agar well diffusion method.

### 2. Materials and Methods

#### 2.1. Preparation of plant extracts

The seeds of *Picrorhiza kurroa* were collected in the month of August from the local field of Lalgudi, Trichy, India. Dried leaves were ground to coarse powder. Powder was first defatted with petroleum ether and then extracted with ethanol which is further evaporated to dryness to obtain alcoholic extract. Aqueous extract were obtained by maceration for 24 hours [4].

#### 2.2. Anti-microbial Assay

Antagonistic action of *Picrorhiza kurroa* extract against *Staphylococcus aureus, Escherichia coli* and *P. aurenginosus* was analyzed by agar well diffusion method as described by Perez et al. (1990) [5]. Aliquot containing plant extract was added to the respective well prepared and petri plates were left undisturbed for 3 hours which leads to diffusion of the sample. Later, the diffused plates were incubated at 30°C for 24-48 hours.

#### 2.3. TLC analysis

The plant sample was subjected to TLC analysis where a mixture of chloroform, methanol and ethyl acetate at ratio of 7:2:1 was used as the mobile phase. The analysed TLC plates were then sprayed with 3, 5- dinitrobenzoic acid in ethanol (2% w/v solution) and KOH in ethanol (6% w/v solution) for visualizing spot position [6].

#### 2.4. FTIR analysis

FTIR analysis were carried out to identify the possible biomolecules responsible for antimicrobial activity using *Picrorhiza kurroa* and the peaks obtained were analyzed by using a standard IR spectra table [7].
3. Results and Discussion

3.1. Anti-microbial Assay

The antibacterial effect of the Picrorhiza kurroa (L) was determined in different concentration such as 0.25mg, 0.50mg and 1mg were recorded as shown in Table 1. The antimicrobial activity was studied against Staphylococcus, Pseudomonas and E.coli. The maximum zone of inhibition(5.0mm) was observed in 1mg concentration against Pseudomonas. The minimum inhibition zone(3.9mm) was observed in same concentration against E.coli. Aqueous extract of Picrorhiza kurroa exhibited enormous activity against Pseudomonas. They were 2.9mm, 3.8mm and 5.0mm zone of inhibition against various concentration of extract. Moderate amount of inhibition zones were recorded in Staphylococcus in same concentration.

Antimicrobial properties are predominant and important tools in diagnosing and controlling the harmful microorganisms during the treatment of infectious diseases and in food spoilage. The active components of natural plant extract with anti-microbial properties usually interfere with growth and metabolism of microorganisms and prevent them from contamination [8].

Table 1: Antimicrobial activity of Picrorhiza kurroa aqueous leaf extracts by Agar well diffusion method.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Plant extract concentration (mg/ml)</th>
<th>Zone of inhibition (mm)</th>
<th>Staphylococcus</th>
<th>Pseudomonas</th>
<th>E.coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.25</td>
<td></td>
<td>2.2</td>
<td>2.9</td>
<td>3.3</td>
</tr>
<tr>
<td>2</td>
<td>0.50</td>
<td></td>
<td>3.4</td>
<td>3.8</td>
<td>3.4</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td></td>
<td>4.4</td>
<td>5.0</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Note: Values are mean of triplicates

3.2. TLC Analysis

TLC analysis suggested the presence of different kinds of phytochemicals in plant extract. Thin layer chromatography was performed on two different extracts using two different solvent systems Methanol: Water: Acetone (18:9:1) for aqueous extract and chloroform: methanol (80:20) for methanolic extract.

TLC of plant extract in aqueous and chloroform reported three spots for various phytochemicals. The reported spots were separated with enough space and having various Rf values showing the presence of at least three phytochemicals in water and chloroform extracts. In this study, the most suitable TLC system for analysis was shown to be chloroform: methanol with the largest discriminating power. Three bands found in this method whose Rf values were 0.42, 0.45 and 0.48.
This values indicate the presence of phenolic compound as shown in Figure 1. This variation in Rf values of the phytochemical compounds is due to their polarity and this polarising property helps in selection of appropriate solvent system which is essential for separation of pure compounds by column chromatography [9]. Solvent mixture with different polarity in various ratio can be used for separation of pure compound from plant extract as shown in Figure 1. This selection of specific solvent for a particular plant extracts can only be performed by analysing the Rf values of compounds in such mixture of solvent system [10].

Figure 1: TLC result of Picrorhiza kurroa plant extract sample.

3.3. FTIR Analysis

FTIR measurement was carried out and its spectrum showed lot of absorption bands indicates the presence of active functional groups in Picrorhiza kurroa as shown in Table 2. The intensity peaks were slightly increased for the period of 3435,2832,2719,2361 cm\(^{-1}\) as well as some intensity peaks decreased like 1362, 997, and 924 cm\(^{-1}\). Figure 2 showed the band at 3435 correspond to O-H Stretching vibrations of alcohol. The peak at 2719 represents to C-H in plane bend to alkenes. The peak at 997 corresponds to C-H, C-Br stretching vibrations to alkyl halides. The weak band at 1045 indicates C-O, C-N stretching vibrations and it corresponds to the presence of alcohols, carboxylic, acids, ethers, esters and aliphatic amines in the plant extract. FTIR spectra showing the presence of IR peaks assigned to polyphenols and also the existence of IR bands characteristic of amide I and amide II groups specific for proteins/enzymes suggest that flavonoids and proteins present in aqueous petal extracts of ornamental plants could be responsible controlling pathogen [11].

Figure 2: FTIR graphical peak analysis of Picrorhiza kurroa plant extract sample.
### Table 2: FTIR analysis showing types of bond present in plant extract sample.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Frequency range</th>
<th>Type of Bond</th>
<th>Type and group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3435.46</td>
<td>O–H stretch, H–bonded</td>
<td>Alcohol, phenols</td>
</tr>
<tr>
<td>2</td>
<td>2832.16</td>
<td>C–H stretch</td>
<td>Alkane</td>
</tr>
<tr>
<td>3</td>
<td>2719.36</td>
<td>C–H-O, C–H stretch</td>
<td>Aldehydes</td>
</tr>
<tr>
<td>4</td>
<td>2361.12</td>
<td>C–H-O, C–H stretch</td>
<td>Aldehydes</td>
</tr>
<tr>
<td>5</td>
<td>2092.40</td>
<td>C–triple bond N–stretch</td>
<td>Carboxyl</td>
</tr>
<tr>
<td>6</td>
<td>1631.09</td>
<td>N–H bend</td>
<td>Primary amine</td>
</tr>
<tr>
<td>7</td>
<td>1133.33</td>
<td>C–H wag (~CH2X)</td>
<td>Alkyl halides</td>
</tr>
<tr>
<td>8</td>
<td>1362.54</td>
<td>C–H rock</td>
<td>Alkane</td>
</tr>
<tr>
<td>9</td>
<td>997.84</td>
<td>C–H bond</td>
<td>Alkenes</td>
</tr>
<tr>
<td>10</td>
<td>924.84</td>
<td>o–H bend</td>
<td>Carboxylic acid</td>
</tr>
</tbody>
</table>

### 4. Conclusion

From the previous research, large number of medicinal plants and their purified compounds from the plant extracts have shown beneficial therapeutic potential. In the present study, TLC analysis of *Picrorhiza kurroa* plant extract revealed the presence of phenolic compound. This study is important in determining the basic biological characterization and imparts the necessity of the compounds identified. The FTIR spectroscopy results also showed that phenolic and aromatic compounds were present in the *Picrorhiza kurroa* plant extract which were similar to the previous findings of phytochemical analysis done by us. Based on FTIR results, we concluded the presence of excessive quantity of aliphatic and aromatic amines and the presence of alkenes in the plant *Picrorhiza kurroa* which may be responsible for its anti-microbial and anti-inflammatory activity.

### References


**Corresponding Author:**

**Jannathul Firdous**,

Email: Jannathul.firdous@unikl.edu.my