# Qualitative And Quantitative Estimation Of Phytoconstituents, Antioxidant And Antimicrobial Activity Of Extract Of Picrorhiza Kurroa

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## Abstract

Green medicine, the drugs derived from plants attained a wide spread interest, as believed to be safe and dependable, compared with costly synthetic drugs that have adverse effects. The present study aimed to analyze a comparative in vitro free radical scavenging and antimicrobial potentials of root extract of Picrorhiza kurroa.

The hydroalcoholic extract of roots Picrorhiza kurroa was studied for antioxidant activity on different in vitro models namely 1,1-diphenyl, 2-picryl hydrazyl (DPPH) assay, Hydrogen peroxide ($H_2O_2$) and Nitric oxide (NO) radical scavenging method. Agar well diffusion method has been used to determine the antimicrobial activities of plant extracts against Enterococcus faecalis, Salmonella Bongori and Klebsiella pneumonia. The extract showed dose dependent free radical scavenging property in the tested models. Picrorhiza kurroa roots extract showed $IC_{50}$ value 78.68µg/ml for DPPH method, which was comparable to that of ascorbic acid ($IC_{50}$=16.35µg/ml). For hydrogen peroxide method, $IC_{50}$ value was found to be 71.93µg/ml, which compares favourable with ascorbic acid ($IC_{50}$=17.16µg/ml). In nitric oxide model, $IC_{50}$ value was found to be 54.94µg/ml, which was comparable to that of ascorbic acid ($IC_{50}$=14.77µg/ml). The extracts exhibited both antibacterial and antifungal activities against tested microorganisms using standard ciprofloxacin (10-30µg/ml). The antimicrobial activity was determined by measuring the diameter of the zone of inhibition in term of millimeter (mm). The antimicrobial activity of hydroalcoholic extract of leaves against all microorganisms was concentration dependent manner but less than standard drug. On the basis of results obtained, it is suggested that both roots of Picrorhiza kurroa extracts may be a potential source of natural antioxidants and antimicrobial compounds to be used in the treatment of various oxidative disorders, infectious diseases caused by resistant microorganisms.



**Keywords:** Picrorhiza kurroa, Hydroalcoholic Extract, Antioxidant activity, Antimicrobial activity.

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## Introduction

Herbals are still used widely by world population, because of better compatibility with the human body and lesser side effect (Rawls; 1996). Therefore, world has now turned its attention to natural products. Picrorhiza kurroa Royle ex Benth. (Scrophulariaceae), is a small perennial herb found mainly in the Himalayan region growing at an elevation of 3,000-5,000 m (Mehra and Jolly; 1968; Subedi; 2000). The leaves of the plant are flat, oval and sharply serrated. The leaf, bark and the underground parts of the plant, mainly rhizomes are widely used in the traditional Indian systems of medicine (Ayurved) since ancient times. Although it shows antioxidant, antiinflammatory and immunomodulatory activities, it is most valued for its hepatoprotective effect. P. kurroa rhizomes are widely used against indigestion problems since ancient times due to improper digestive secretions (Krishnamurthy; 1969). The major glycoside is 'Kutkin', which is a mixture of (picroside-I and II) and possess

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significant hepatoprotective action (Shukla et al., 1991). The major uses of the plant are due to its hepatoprotective, anticholestatic, and immunomodulatory activity (Nadkarni; 1954; Ansari et al., 1988; Chaturvedi and singh; 1966; Langer et al., 1981; Dey et al., 1980). Literature reviews pointed out that no studies combining the antioxidant and antimicrobial activities of the root extract of Picrorhiza kurroa have so far been undertaken. Coupled with our continuous interest of pharmacological screening of Indian medicinal plants, in this study we aimed to investigate the antioxidant and antimicrobial activities of the root extract of Picrorhiza kurroa.

## Material and Methods

### Material

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade. The pathogenic bacteria and fungus used in the current study obtained from Microbial Culture collection, National Centre forcell science, Pune, Maharashtra, India.

### Methods

### Procurement of Plant Material

Root of Picrorhiza kurroa were collected from Bhopal. After the plant was collected, they have been processed for cleaning in order to prevent the deterioration of phytochemicals present in plant.

### Powdering

The dried plant part was finely powdered using electric grinder, sieved and packaged in polyethylene bags until when needed.

### Extraction procedure

Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs (Khandelwal, 2005; Kokate, 1994).

### Successive extraction with different solvents by maceration method

60 gram plant material were extracted in three solvents of different polarity viz ethanol, hydroalcoholic (ethanol: water in ratio of 70:30) and water (Mukherjee, 2007). Powdered plant materials were extracted by maceration method. The resultant content was filtered with Whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for analysis.

### Determination of extractive value (% yield)

### Calculation of % yield

The % yield of yield of each extract was calculated by using formula:

$$\textbf{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

### Qualitative phytochemical analysis

**1. Detection of alkaloids:** Extracts dissolved individually in dilute Hydrochloric acid and filtered.

**a) Hager's Test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.

**2. Detection of carbohydrates:** Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

**a) Fehling's Test:** Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

**3. Detection of glycosides:** Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

**a) Legal's Test:** Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Finding of pink to blood red colour indicates the presence of cardiac glycosides.

**4. Detection of saponins**

**a) Froth Test:** Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the incidence of saponins.

**5. Detection of phenols**

**a) Ferric Chloride Test:** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

**6. Detection of flavonoids**

**a) Lead acetate Test:** Extracts were treated

with few drops of lead acetate solution. Formation of yellow colour precipitate indicate the occurrence of flavonoids.

**7. Detection of proteins**

**a) Xanthoproteic Test:** The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

**8. Detection of diterpenes**

**a) Copper acetate Test:** Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicate the presence of diterpenes (Roopashree et al., 2008; Obasi et al., 2010; Audu et al., 2007).

## Quantitative studies of phytoconstituents

### Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Olufunmiso and Anthony, 2011). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

### Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Olufunmiso and Anthony, 2011). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

### In-vitro antioxidant activity using different methods

### DPPH method

DPPH scavenging activity was measured by the spectrophotometer (Olufunmiso et al., 2011). Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance (Olufunmiso and Anthony, 2011). Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

$$\text{Calculation of \% Reduction} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{X } 100$$

### Nitric oxide method

Nitric oxide was produced from sodium nitroprusside and the Griess reagent was measured. Sodium nitroprusside spontaneously produces nitric oxide in aqueous solution at physiological pH, interacting with oxygen to generate nitric ions that can be estimated using Griess reagent. Nitric oxide scavengers compete with oxygen resulting in decreased nitric oxide manufacturing (Marcocci et al., 1994). Sodium nitroprusside (10 mmol / L) was mixed with various extract concentrations in phosphate buffer saline (PBS) and incubated at 25°C for 150 min. Griess reagent (1% sulphanilamide, 2% $H_3PO_4$ and 0.1% naphthyl ethylenediamine dihydrochloride) was added to the specimens. The chromophore absorbance created during the diazotization of sulphanilamide nitrite and subsequent coupling with naphthyl ethylenediamine was read at 546 nm and referred to the absorption of conventional ascorbic acid solutions treated in the same manner with Griess reagent as a positive control. All triplicate experiments were conducted and the chart was plotted with the mean values. The inhibition proportion was evaluated using the following formula:

Radical scavenging activity (%) = $(A_{control}-A_{test})/A_{control}\times100$

Where Acontrol is the absorption (without

extract) of the control and where Atest is the absorption in the presence of the extract / standard.

## Hydrogen Peroxide method

In-vitro antioxidant activity of extract of Picrorhiza kurroa using hydrogen peroxide was performed as Czochra and Widensk (2002) proposed. Added 2ml hydrogen peroxide (43 mol) and 1.0 ml ethanol sample [20-100 µl different extracts (4 mg / ml) ethanol] accompanied by 2.4 ml 0.1 M phosphate buffer (pH 7.4). The resulting solution was maintained for 10 minutes and the absorbance at 230 nm was recorded. Three times all measurements were repeated. Without adding hydrogen peroxide, blank was ready and control was prepared without sample. It was used as a conventional compound with ascorbic acid. Free radical hydrogen peroxide scavenging activity (percent) has been calculated.

## Antimicrobial activity of Picrorhiza kurroa extract

Nutrient agar (NAM) and potato dextrose (PDA) media was prepared for growing of microbes inside the laboratory. The standard size (100mm× 15mm) petri dishes as required for whole experiment. For preparation of media, 28 gram NAM powder and 24 gram PDA powder was mixed with 1000 ml of distilled water and stirred to obtain homogenized mixture respectively. After which, NAM and PDA mixture was placed in Autoclave under 15 psi pressure, at 121°C for 25 min for sterilization of media. After that poured the culture media into petri dishes at ratio of 20 ml/dish and was left half covered on the table to let the agar cool down and solidify at room temperature.

Antimicrobial activity of Picrorhiza kurroa extract was determined, using the agar well diffusion assay method as described by Holder and Boyce. The plates were done in triplicates and were incubated at 37°C. The antimicrobial activity was taken on the basis of diameter of zone of inhibition, which was measured after 2 days of incubation and the mean of three readings is presented. The extract and the standard were dissolved in distilled water.

**Statistical analysis:** Each experiment has three replicates and three determinations were conducted. Means of variable and standard deviation were recorded.

## Results and Discussion

The crude extracts so obtained after each of the successive extraction with ethanolic, hydroalcoholic and aqueous were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of ethanolic, hydroalcoholic and aqueous extracts obtained from the roots of Picrorhiza kurroa were found to be 8.4%, 22.2% and 18.5% w/w respectively.

Total phenolic compounds (TPC) were expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $y = 0.015x + 0.011$, $R^2 = 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $y = 0.037x + 0.016$, $R^2 = 0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance. The Hydroalcoholic extract of roots of Picrorhiza kurroa was found 0.493 and 0.435mg/100mg of total phenolic and flavonoids contents respectively. The extract showed dose dependent free radical scavenging property in the tested models. Picrorhiza kurroa roots extract showed $IC_{50}$ value 78.68µg/ml for DPPH method, which was comparable to that of ascorbic acid ($IC_{50}$=16.35µg/ml). For hydrogen peroxide method, $IC_{50}$ value was found to be 71.93µg/ml, which compares favourable with ascorbic acid ($IC_{50}$=17.16µg/ml). In nitric oxide model, $IC_{50}$ value was found to be 54.94µg/ml, which was comparable to that of ascorbic acid ($IC_{50}$=14.77µg/ml). The extracts exhibited both antibacterial and antifungal activities against tested microorganisms using standard ciprofloxacin (10-30µg/ml). The antimicrobial activity was determined by measuring the diameter of the zone of inhibition in term of millimeter (mm). The antimicrobial activity of hydroalcoholic extract of leaves against all microorganisms was concentration dependent manner but less than standard drug. On the basis of results obtained, it is suggested that both roots of Picrorhiza kurroa extracts may be a potential source of natural antioxidants and antimicrobial compounds to be used in the treatment of various oxidative disorders, infectious diseases caused by resistant microorganisms.

**Table 1.1:** Extractive values of Picrorhiza kurroa

| S. No. | Extracts | % Yield* (W/W) |
|---|---|---|
| 1. | Ethanolic | 8.4% |
| 2. | Hydroalcoholic | 22.2% |
| 3. | Aqueous | 18.5% |

**Table 1.2:** Result of phytochemical screening of extract of Picrorhiza kurroa using maceration method

| S. No. | Constituents | Ethanolic extract | Hydroalcoholic extract | Aqueous extract |
|---|---|---|---|---|
| 1. | **Alkaloids** Hager's Test: | -ve | -ve | -ve |
| 2. | **Glycosides** Legal's Test: | -ve | -ve | -ve |
| 3. | **Flavonoids** Lead acetate Test: Alkaline test: | -ve +ve | +ve -ve | +ve +ve |
| 4. | **Diterpenes** Copper acetate Test: | +ve | -ve | +ve |
| 5. | **Phenol** Ferric Chloride Test: | +ve | +ve | -ve |
| 6. | **Proteins** Xanthoproteic Test: | +ve | +ve | -ve |
| 7. | **Carbohydrate** Fehling's Test: | +ve | +ve | -ve |
| 8. | **Saponins** Froth Test: | +ve | +ve | +ve |
| 9. | **Tannins** Gelatin test: | -ve | -ve | -ve |

+ve= present, -ve=negative

**Table 1.3:** Estimation of total phenol and flavonoids content of Picrorhiza kurroa

| S. No. | Extracts | Total phenol content | Total flavonoids content |
|---|---|---|---|
| | | (mg/ 100 mg of dried extract) | |
| 1. | Ethanolic | 0.324 | 0.386 |
| 2. | Hydroalcoholic | 0.493 | 0.435 |
| 3. | Aqueous | - | 0.263 |

**Table 1.4:** % Inhibition of ascorbic acid and extract of Picrorhiza kurroa using DPPH method

| S. No. | Concentration (µg/ml) | Percentage Inhibition | |
|---|---|---|---|
| | | Ascorbic acid | Extract |
| 1 | 10 | 40.52 | 22.58 |
| 2 | 20 | 56.52 | 22.99 |
| 3 | 40 | 64.52 | 30.10 |
| 4 | 60 | 71.36 | 38.35 |
| 5 | 80 | 80.85 | 51.61 |
| 6 | 100 | 88.67 | 61.64 |
| **IC 50** | | **16.35** | **78.68** |

**Table 1.5:** % Inhibition of ascorbic acid and extract of Picrorhiza kurroa using Hydrogen Peroxide method

| S. No. | Concentration (µg/ml) | Percentage Inhibition | |
|---|---|---|---|
| | | Ascorbic acid | Extract |
| 1 | 20 | 43.63 | 22.63 |
| 2 | 40 | 67.23 | 38.52 |
| 3 | 60 | 77.42 | 47.52 |
| 4 | 80 | 80.56 | 53.64 |
| 5 | 100 | 85.75 | 60.58 |
| **IC 50** | | **17.16** | **71.93** |

**Table 1.6:** Percentage Inhibition of ascorbic acid and extract of Picrorhiza kurroa using NO method

| S. No. | Concentration (µg/ml) | Percentage Inhibition | |
|---|---|---|---|
| | | Ascorbic acid | Extract |
| 1 | 20 | 45.65 | 26.65 |
| 2 | 40 | 69.98 | 45.58 |
| 3 | 60 | 79.98 | 53.32 |
| 4 | 80 | 85.65 | 65.45 |
| 5 | 100 | 92.54 | 73.32 |
| **IC 50** | | **14.77** | **54.94** |

**Table 1.7:** Antimicrobial activity of standard drug against selected microbes

| S.N | Name of drug | Microbes | Zone of Inhibition (nm) | | |
|---|---|---|---|---|---|
| | | | 30 µg/ml | 20 µg/ml | 10 µg/ml |
| 1. | Ciprofloxacin | Enterococcus faecalis | 22±0.74 | 18±0.47 | 13±0.57 |
| | | Salmonella Bongori | 25±0.5 | 23±0.86 | 17±0.15 |
| | | Klebsiella pneumoniae | 36±1.699 | 28±1.24 | 19±4.71 |

(n=3, Mean±SD)

**Table 1.8:** Antimicrobial activity of hydroalcoholic extract of Picrorhiza kurroa against selected microbes

| S. No. | Name of microbes | Zone of inhibition (mm) | | |
|---|---|---|---|---|
| | | 100mg/ml | 50 mg/ml | 25mg/ml |
| 1. | Enterococcus faecalis | 8±0.47 | 7±0.47 | 6±0.47 |
| 2. | Salmonella Bongori | 8±0.47 | 7±0.47 | 6±0.47 |
| 3. | Klebsiella pneumoniae | 9±0.47 | 8±0.47 | 7±0.47 |

(n=3, Mean±SD)

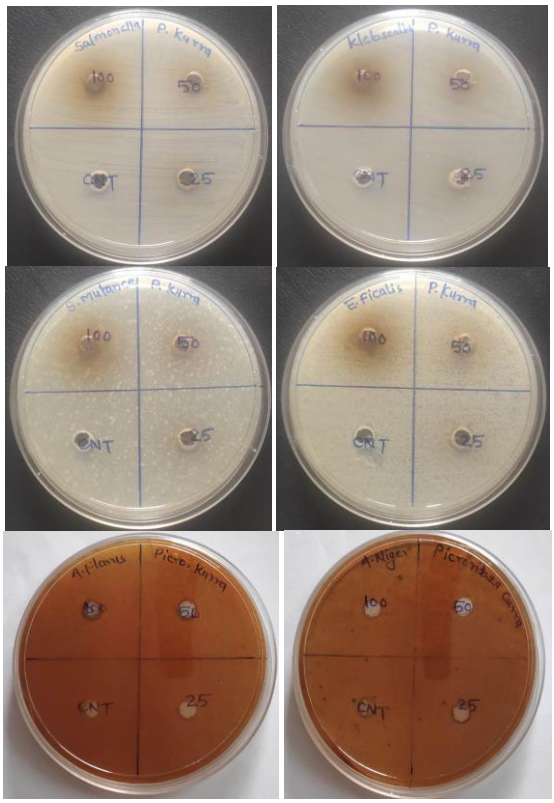

**Figure 1:** Antimicrobial activity of hydroalcoholic extract of Picrorhiza kurroa against selected microbes

## Conclusion

From the experiment it has shown that hydroalcoholic extracts have been used in vitro to inhibit the growth of some disease-causing bacteria. It can therefore be suggested that plant extracts have great potential as antimicrobial compounds against micro-organisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms. They can also be a source of natural antioxidants agents. Due to their antibacterial and antioxidant activities Picrorhiza kurroa extracts have promising potential as a source of natural antioxidant and antimicrobial agents. Therefore, these results

are encouraging enough to pursue characterization of these fractions in different other models in detail. Further studies may also be conducted to isolate & purify the active constituents to evaluate others activity.

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