



Preliminary Phytochemical screening and Antioxidative potential of *Picrorhiza kurroa* Royale ex. Benth

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ABSTRACT

Picrorhiza kurroa, commonly known as kutki and belongs to the family Scrophulariaceae. It is an herbal plant native to the Himalayan regions of India, Pakistan, and Nepal. It is reported to be an effective hepatoprotective and anti-neoplastic plant that also possesses antihyperlipidemic, immunomodulatory and antioxidant activities. *Picrorhiza kurroa* is rich in several phytochemicals and thus the present study was planned to explore its antioxidant potential through *in vitro* assays. In the present study fifty percent hydromethanolic extract of rhizome and roots of *Picrorhiza kurroa* (PKE) was prepared and analyzed for presence of various phytoconstituents in PKE employing different qualitative and quantitative biochemical analyses. The extraction yield of hydromethanolic extract of PKE was found to be 7.78%. Biochemical analyses revealed presence of various phytoconstituents in PKE viz., resins, Saponins, flavonoids, alkaloids, glycosides, etc. The total phenolics content of PKE was estimated to be 544mg/g while total flavonoids content of PKE was found to be 400 mg/g. In DPPH assay, IC_{50} of PKE was found to be 153.56 μ g/ml where as in Nitric oxide scavenging assay IC_{50} of PKE was found to be 86.82 μ g/ml. Thus it could be inferred that PKE displayed presence of various phytoconstituents which could be responsible for its antioxidative potential and medicinal value.

Key words: *Picrorhiza kurroa*, phytochemical analysis, flavonoids, phenolics.

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INTRODUCTION

There are plenty of antioxidant substances present in plants in the form of phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignins, tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, [7, 2]. So to maintain a healthy body, one should always increase the intake of foods rich in antioxidant compounds that lower the risk of chronic health problems associated with the above disease conditions [8]. Many antioxidant compounds, naturally occurring in plant sources, have been identified as a free radical or active oxygen scavengers.

Picrorhiza kurroa Royle ex Benth (kutki) grows at an altitude of 3000 to 5000 meters in the Himalayan regions. It is a small important alpine herb. Leaves are radical, spatulate sharply serrated, flowers white or pale blue-purple in dense terminal spicate raceme. This plant has been traditionally used to treat liver and upper respiratory tract disorders. It is also used to reduce fever and treat dyspepsia, chronic disorders and scorpion sting. The root of this plant is used as traditional medicine in India. A wide range of biological properties such as cardiovascular, antihepatotoxic, choleric, hypoglycemic, antiinflammatory, antiposmodic, antitumor, antioxidant, mulluceicidal and leishmanicidal activities has been showed by this plant [3, 4, 15]. *Picrorhiza kurroa* is reported to possess various phytochemical slikepicroside I, II, D-mannitol, kutkiol, kutki sterol and apocynin [20]. One of the active metabolites of *Picrorhiza kurroa*, flavonoid apocynin has been reported to attenuate Parkinson's, hypoxia and ischemia-reperfusion by its inhibitory action on NADH oxidase; expressed during oxidative stress [21, 18, 14, 9]. Thus the present study was conducted to explore presence of various phytochemicals in fifty percent hydromethanolic extract (PKE) through qualitative and quantitative phytochemical analysis.

Further the antioxidative potential of PKE was evaluated through *in vitro* DPPH and nitric oxide radical scavenging assays.

MATERIAL AND METHODS

Preparation of plant extract

The PKE was prepared as per the method described by Deb *et al.*, [5] for the preparation of *Picrorhiza kurroa* root and rhizomes extract (PKE). The percent yield was calculated by dividing quantity of the plant extract obtained by 50gm of dry powder. The prepared extract was weighed, kept in airtight containers and stored at -20°C till further use.

Qualitative analyses

Qualitative tests were carried out to detect the presence of phenolics, flavonoids, tannins, alkaloids, saponins, carbohydrate, protein, amino and phytosterol in the plant extract as per the method described by [5, 19].

A. Phenolics:

- **Ferric Chloride Test** - 500 mg of extract was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds

B. Flavonoids:

- **Ammonia Test** - A few drops of 1% ammonia solution was added to the extract of plant sample in a test tube. A yellow coloration shows the presence of flavonoid compounds in the sample.
- **H₂SO₄ Test** - 50 mg of the extract was taken in test tube and was treated with a few drops of sulphuric acid. Change in colour of the solution to orange indicates the presence of several flavanoids in the sample.
- **Alkaline reagent Test** - 50 mg of the plant extract was taken in test tube and a few drops of sodium hydroxide was added to each tube. Formation of yellow colour, which on addition of dilute acid, becomes colourless, shows the presence of flavanoids in the extract.

C. Tannins:

- **Gelatin Test** - To 20 mg of the plant extract, 1% gelatin solution was added. Formation of white precipitate is an indication for the presence of tannins in the sample.

D. Phytosterols:

- **Salkowski's Test** - Small quantity of the extract was dissolved in 5ml of chloroform separately. To 1ml of above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Presence of brown colour shows the presence of phytosterols in the plant extract.

E. Alkaloids:

- Small quantity of the extract was separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were used for the test with Dragendroffs reagent. The appearance of orange brown precipitate indicates the presence of alkaloids.

F. Saponins:

- **Foam test** - 0.5 gm of the plant extract was taken in 2 ml vials and was dissolved in 2 ml of water. The sample was shaken vigorously for 10-15 seconds. If the foam produced persists for 10 minutes, saponins are present in the sample.

G. Proteins:

- **Xanthoprotic Test** - To the plant extract, a few drops of nitric acid was added. Formation of yellow colour shows the presence of proteins in the plant extract.

H. Amino acids:

- **Ninhydrin Test** - To the plant extract, few drops of 25% ninhydrin solution was added. Then the solution was boiled for few minutes. Change in colour of the solution to blue, indicates the presence of amino acids.

I. Carbohydrates:

The plant extract was dissolved in 5 ml distilled water in test tubes to carry out following tests.

- **Molisch's Test** - To above solutions, 2-3 drops of alcoholic α naphthol solution was added. Formation of violet ring shows the presence of carbohydrates.
- **Benedict's test** - The above solutions of plant extract was treated with Benedict's reagent and heated. Formation of orange precipitate shows the presence of carbohydrates.

Quantitative analyses of the plant extract

Qualitative analysis for determination of total phenolic and total flavonoid content present in PKE was carried out. Plant's total phenolic content was determined by the method described [5]. Plant's flavonoid content was determined by following the aluminium chloride colorimetric assay described by [5].

Evaluation of antioxidative potential of the plant extract

DPPH radical - scavenging assay

Scavenging of stable DPPH radical was assayed *in vitro* as per the method described by Shivhare *et al.* [17]. This assay was performed to evaluate antioxidative potential of the plant extract.

$$\text{Percentage (\%)} \text{ of DPPH radical scavenging} = \frac{(\text{Abs of negative control} - \text{Abs of test sample})}{\text{Abs of negative control}} \times 100$$

The IC₅₀ values the extract was obtained by regression analysis of the corresponding dose-response curve.

NO radical - scavenging assay

The antioxidant activity of the extract was measured on the basis of the scavenging activity of free radical according to the method described by Jagetia *et al* [11].

RESULTS AND DISCUSSION

Percentage yield

The percentage yield of hydromethanolic extract of *Picrorhiza kurroa*(PKE) was found to be 7.78%.

Phytochemicals analyses of PKE

Various biochemical tests for detection of different phytochemicals in PKE were conducted. Estimation of total phenolic and flavonoid content in PKE was also carried out.

Qualitative tests for phytochemicals

As per the biochemical tests conducted, PKE showed presence of almost all the tested phytochemicals, viz. flavonoids, alkaloids, steroids, phenols, etc. (Table 1).

Quantitative tests for phytochemicals

Estimation of total phenolic content in PKE

Total phenolic content was reported as mg / gm of extract gallic acid equivalent (GAE) as presented in Table 2. The total phenolic content of the PKE was estimated to be 544mg/g.

Estimation of total flavonoid content in PKE

The total flavonoids content was represented as mg rutin equivalent (RE) per g of extract with reference to standard curve as presented in Table 3. Total flavonoids content of the PKE was found to be 400mg/g.

DPPH radical - scavenging assay

In order to explore the antioxidative potential of PKE, DPPH radical scavenging assay was performed and percentage scavenging for PKE and ascorbic acid was calculated. An increase in percentage scavenging was observed with increase in concentration of PKE and ascorbic acid (Table 4 and Figure 1). IC₅₀ of PKE was found to be 153.56 µg/ml.

Nitric Oxide scavenging assay

The results showed that percentage scavenging activity of PKE and ascorbic acid was increased in a dose dependent manner. The results are depicted in Table 5 and Figure 2. IC₅₀ of PKE was found to be 86.82µg/ml.

Herbal remedies have been extensively used since times immemorial throughout the world. These have wide acceptability due to their time-tested therapeutic values and minimal side effects in contrast to modern allopathic medicines. Medicinal plants have various phytochemicals and constituents which are secondary metabolites responsible for their antioxidative potential [2]. Although *Picrorhiza kurroa* have showed antioxidant, antiinflammatory and immunomodulatory activities, it is most valued for its hepatoprotective effect [21]. *Picrorhiza* is a potent modulator of the immune system and presents a strong hepatoprotective action [10]. PKE exhibited significant antioxidative potential as it had good DPPH and NO radical scavenging activity. The higher percentage scavenging of free radicals by the extract reduces the cell damage by free radicals. Flavonoids and their derivatives have capacity to act as antioxidants that can protect the body against reactive oxygen species [1]. The phenolic content of *Picrorhiza kurroa* root ethanolic extract was reported to be 222 ± 11.4 µg GAE/mg extract, whereas the flavonoid content was 197 ± 9.8 µg quercetin equivalents/mg extract. Kant *et al.* [13] reported antioxidant activity in leaves of *Picrorhiza kurroa*. Deshpande *et al.* [6] reported antioxidant effects of *Picrorhiza kurroa* rhizome extracts in alcoholic cirrhosis of liver. The methanol extracts of *Picrorhiza kurroa* rhizomes significantly reduced oxidative stress and elevated antioxidants which were measured spectrophotometrically. The antioxidant and anti-neoplastic activities of methanolic and aqueous extracts of *Picrorhiza kurroa* rhizome were investigated by Rajkumar *et al.* [15] Both the extracts exhibited promising antioxidant potentials. The extracts were also observed to be cytotoxic at the tested dosage and were able to target cells towards apoptosis. Overall *Picrorhiza kurroa* is reported to possess good antioxidant potential and could be used as food supplement [12].

Thus considering the antioxidant activity of PKE, it is worthwhile to explore it further for development of plant based preventive and therapeutic drug. However, the extract should also be evaluated for other pharmacological effects in suitable *in vivo* and *in vitro* system.

Table 1- Qualitative test of hydromethanolic plant extract of *Picrorhiza kurroa*

S. No.	Phytochemical Analysis		Extract of <i>Picrorhiza kurroa</i> (PKE)
1.	Phenolics	Ferric Chloride Test	+
		Lead acetate Test	+
2.	Flavonoids	Ammonia Test	+
		H ₂ SO ₄ Test	+
		Alkaline reagent Test	+
3.	Tannins	Gelatin Test	-
4.	Phytosterols	Salkowski's Test	+
5.	Alkaloids	Dragendroffs reagent test	+
6.	Saponins	Foam test	+
7.	Protein	Xanthoprotic Test	+
8.	Amino acids	Ninhydrin Test	-
9.	Carbohydrate	Molisch's Test	+
		Benedict's test	+

Table 2: Total phenolics content in PKE

S. No.	Plant extract	Concentration of extract ($\mu\text{g/ml}$)	O.D. (765nm)	Total phenolics content ($\mu\text{g}/\mu\text{g}$ extract)	Total phenolics content (GAEmg/g extract)
1.	<i>Picrorhiza kurroa</i> (PKE)	5	0.051	0.544	544

Table 3: Total flavonoid content in PKE

S. No.	Plant extracts	Concentration of extract ($\mu\text{g/ml}$)	O.D. (510nm)	Total phenolics content ($\mu\text{g}/\mu\text{g}$ extract)	Total phenolics content (RE mg/g extract)
1.	<i>Picrorhiza kurroa</i> (PKE)	5	0.035	0.40	400

Table 4: DPPH radical - scavenging assay

S. No.	Concentrations used ($\mu\text{g/ml}$)	Mean O.D. of PKE (517nm) \pm SE	Percent Scavenging of PKE	Mean O.D. of ascorbic acid \pm SE	Percent Scavenging of Ascorbic acid
1	1	0.760 \pm 0.027	21.33 \pm 2.83	0.645 \pm 0.041	33.26 \pm 4.26
2	5	0.743 \pm 0.028	23.09 \pm 2.93	0.631 \pm 0.010	34.74 \pm 1.13
3	10	0.723 \pm 0.049	25.23 \pm 5.13	0.554 \pm 0.042	42.7 \pm 4.39
4	20	0.675 \pm 0.005	30.16 \pm 0.538	0.489 \pm 0.087	49.43 \pm 9.02
5	40	0.639 \pm 0.127	32.19 \pm 1.69	0.422 \pm 0.061	56.35 \pm 6.38
6	60	0.593 \pm 0.028	33.12 \pm 2.91	0.4 \pm 0.073	58.62 \pm 7.59
7	80	0.646 \pm 0.046	38.64 \pm 4.78	0.381 \pm 0.071	60.59 \pm 7.42
8	100	0.592 \pm 0.021	38.77 \pm 2.18	0.344 \pm 0.061	64.42 \pm 6.31
9	Control	0.967 \pm 0.002			

* Significant at P<0.05

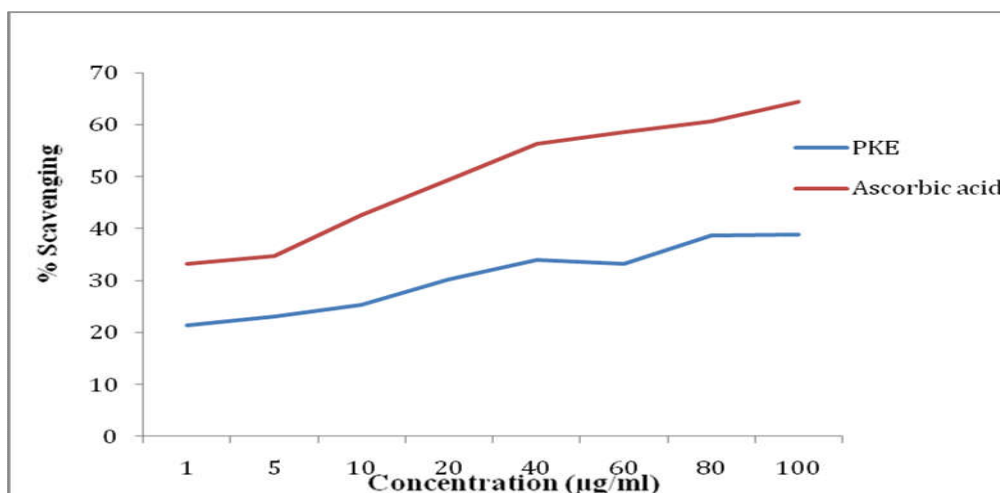


Figure 1: Percent scavenging of PKE and ascorbic acid in DPPH assay

Table 5: Nitric Oxide scavenging assay

S.No.	Concentrations used (µg/ml)	Mean O.D. of PKE ± SE	Percent Scavenging of PKE	Mean O.D. of ascorbic acid ± SE	Percent Scavenging of Ascorbic acid
1	10	0.356±0.007	28.942±1.40	0.409±0.001	13.77±0.239
2	20	0.286±0.004	42.914±0.806	0.373±0.001	18.56±0.115
3	40	0.277±0.001	44.710±0.115	0.321±0.002	25.14±0.399
4	60	0.271±0.008	45.908±1.72	0.393±0.001	33.73±0.066
5	80	0.271±0.005	45.908±1.15	0.442±0.001	40.71±0.176
9	Control	0.501±0.001			

* Significant at P<0.05

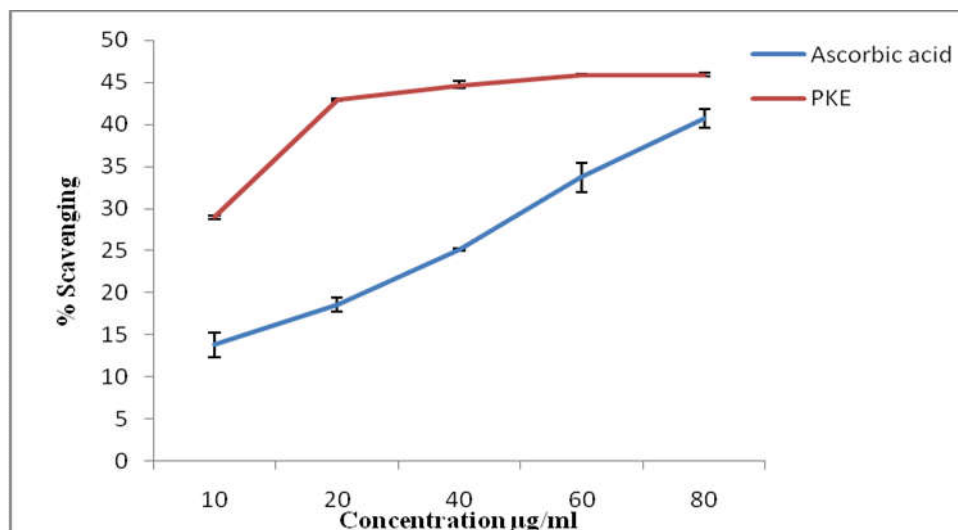


Figure 2: Percent scavenging of NO

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