

QUALITATIVE ANALYSIS OF *BERBERIS* AND *PICRORHIZA KURROA*Satya Prakash Maurya^{1*}, Amrita Asthana², Somendra Kumar Maurya³, Pooja Maurya⁴, Kaushal Yadav⁵ and Anita Maurya⁶¹Department of Pharmacy, Academic Head of R.D.S College of Pharmacy, Jaunpur 222136.^{2,3,4,6}Department of Pharmacy, Assistant Professor, Prasad Institute of Technology, Jaunpur U.P 222001⁵Assistant Professor, R.D.S College of Pharmacy, Jaunpur 222136.***Corresponding Author: Satya Prakash Maurya**

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ABSTRACT

The genus *Berberis* has an important place in various traditional systems of medicine worldwide for their efficacious medicinal properties. The bark is used as a tonic and anti-periodic. This plant is also well proven for cardio vascular, hepato-protective, antimicrobial and anti-cancerous activities. *Picrorhiza kurroa* is Natural products have been an important resource for the maintenance of life for ages. Already in the earliest written traditions. It is used in all forms of liver damage, cirrhosis and inflammation of the liver. It protects the liver against damage from the hepatitis C virus. The extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are carbohydrates, flavonoids, alkaloids, glycosides, steroids are present in *B. aristata* and *Picrorhiza kurroa* may carbohydrate, steroid, cardiac glycoside and flavonoids are present.

KEYWORD: *Berberis*, *Picrorhiza kurroa*.**INTRODUCTION**

The genus *Berberis* has an important place in various traditional systems of medicine worldwide for their efficacious medicinal properties. The ancient Ayurvedic literature of India records uses of *Rasaut* (*Ras*-juice; *out*-frothing and foaming when boiling; hence *Rasaut* means concentrated juice), an extract of either stem or root of *Berberis* sp. The specific uses of *Rasaut* for curing eye diseases and indolent ulcers earned a great fame. The roots of *Berberis* species are employed as an anti-periodic, diaphoretic and antipyretic, and its action was believed to be as powerful as quinine. The bark is used as a tonic and anti-periodic. This plant is also well proven for cardio vascular, hepato-protective, antimicrobial and anti-cancerous activities. *Berberis aristata* is characterized by an erect spiny shrub, ranging between 2 to 3 m (6.6 to 9.8 ft) in height. It is a woody plant, with bark that appears yellow to brown from the outside and deep yellow from the inside. The bark is covered with three-branched thorns, which are modified leaves, and can be removed by hand in longitudinal strips. The leaves are arranged in tufts of five to eight and are approximately 4.9 cm (1.9 in) long and 1.8 cm (0.71 in) broad. The leaves are deep green on the dorsal surface and light green on the ventral surface. The leaves are simple with pinnate venation. The leaves are leathery in texture and are toothed, with several to many small indentations along the margin of the leaf.^[1]

Scientific Classification**Kingdom:** Plantae**Clade:** Angiosperms**Clade:** Eudicots**Order:** Ranunculales**Family:** Berberidaceae**Genus:** *Berberis***Species:** *B. aristata**B. aristata***Uses**

The fruits of the species are eaten by people living in areas where the plant is found, often as a dessert. They are juicy and contain plenty of sugars and other useful nutrients that supplement their diet. The roots can also be used for making an alcoholic drink. The plant as a whole is a good source of dye and tannin which is used for dyeing clothes and for tanning leather.^[2]

Medicinal uses

B. aristata is used in traditional herbal medicine. Its stem, roots, and fruits are used in Ayurveda.^[2,3] A preparation called rasaunt is prepared by boiling the bark of the root and of the lower part of the stem in water. The solution is then strained and evaporated until a semi-solid mass, rasaunt, is obtained. It is mixed with either butter or alum or with opium and lime-juice.^[1]

The root bark contains the bitter alkaloid berberine, which has been studied for its potential pharmacological properties.

2. *Picrorhiza kurroa*

Natural products have been an important resource for the maintenance of life for ages. Already in the earliest written traditions e.g. the Rig-Veda of South Asia (1500-900 BC), it is evident that plants played an important role in daily life. One of the best-known examples is Soma, a plant that was pressed to yield juice, which was used as a medicine. The interest in medicinal plants has never ceased since even today, natural products become increasingly important as a source of pharmacotherapeutics.^[4]

Picrorhiza kurroa is one of the major incomes generating non-timber forest products found in the Nepalese Himalayas. It is one of the oldest medicinal plants traded from the Karnali zone. Known as Kutki in Nepali, it is a perennial herb and is used as a substitute for Indian gentian (*Gentiana kurroo*).^[5]

Scientific classification

Kingdom: Plantae

Clade: Angiosperms

Clade: Eudicots

Order: Lamiales

Family: Plantaginaceae

Genus: *Picrorhiza*

Species: *P. kurroa*

Synonyms of *Picrorhiza kurroa*: In Sanskrit it is known as Tikta, Tikta rohini, Katurhini, Sutiktaka, Kauka; in Assamese it is called as Katki, Kutki; in English it is known as Black Hellebore,^[6] Hellebore,^[7,8] Yellow gentian,^[9] in Gujarati it is known as Kadu, Katu; in Hindi it is called Kutki. This plant also known as Katuka rohini, katuka rohini in Kannada; Kutki, Kalikutki in Marathi; Katuki in Oriya; Karru, kaur in Punjabi; Katuka rohini, Katuku rohini, Kadugurohini in Tamil.^[10,11,12,13]



Picrorhiza kurroa

Description

Leaves: 5–15 cm long leaves, almost all at the base, often withered.^[2] Leaves are coarsely toothed, narrowed to a winged stalk.^[5]

Rhizomes of the plant are 15–25 cm long and woody.

Flowers: small, pale or purplish blue, borne in cylindrical spikes, spikes borne on almost leafless erect stems. Flowers about 8 mm, 5-lobed to the middle, and with much longer stamens.

Fruits: 1.3 cm long.

Chemistry: Chemical composition of *Picrorhiza kurroa* include Kutkin, a bitter glycoside which contains two C-9 iridoid glycosides-Picroside I and Kutakoside.^[14]

Chemical Constituents of *Picrorhiza kurroa*: The therapeutically potent constituents of the drug essentially comprises of three vital bitter glycosides, namely: Picroside I, Picroside II and Kutkoside. Among them chemically both Picroside and Kutkoside are C-9 monoterpenes. Iridoid glycosides having an epoxy moiety present in the cyclopentane ring. Besides, it also contains organic acids, resin, sugar and tannins along with cucurbitacin glycosides (highly oxygenated triterpenes), apocynin androsin, D-mannitol, Kutkiol, Kutkisterol, Apocyanin, Phenol glucosides, Androsin, and Picein Iridoid glycosides, Kutkin, Picroside I, II, III, IV, V, Kutkoside, Picrorhizin.^[7,8,9,10,11]

Uses

The rhizome has a long history of use in Indian Ayurvedic medicine for the treatment of digestive problems. Other uses have been proposed (e.g. for asthma, liver damage, wound healing, vitiligo) but the medical evidence is not yet conclusive. It appears to be relatively safe based on its long history of traditional use.^[15,16] Our study Arka Aayurveda Current research on *Picrorhiza kurroa* has focused on its hepatoprotective, anticholestatic, antioxidant, and immune-modulating activity. Kutki has hepato-protective properties and thus supports the liver and spleen. It is used in all forms of liver damage, cirrhosis and inflammation of the liver. It

protects the liver against damage from the hepatitis C virus.

Traditional uses: This plant is used as Svasa, Daha, Jvara, Kamala, Kustha, and Arocaka.^[7]

Modern uses: The dried roots & rhizomes are used as hepatoprotective, antiasthmatic, immunomodulatory agent particularly for liver disorders & jaundice, fever, dysentery and diarrhea.^[8,9]

2. MATERIAL AND METHOD

2.1 Collection of Plant Material

The leaves of *Berberis* and *Picrorhiza Kurroa* collected from Botanical garden of RDS College of Pharmacy.

3. Qualitative Chemical Investigation of Extracts

3.1 Photochemical screening of extracts

Photochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are carbohydrates, flavonoids, alkaloids, glycosides, steroids and fats, tannins and phenolic compounds.

4. Tests for Carbohydrates

4.1. (Molish's test General test)

Took 2-3 ml aqueous extract, added few drops of naphtha solution in alcohol, shaken and added concentrated H₂SO₄ from sides of the test tube was observed for violet ring at the junction of two liquids.

4.1.1 Fehling's test: 1 ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one minute. Added equal volume of test solution. Heated in boiling water bath for 5-10 min was observed for a yellow, then brick red precipitate.

4.1.2 Benedict's test: Equal volume of Benedict's reagent and test solution in test tube were mixed. Heated in boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in test solution.

4.2. Tests for Monosaccharides

4.2.1 Barfoed's test: Equal volume of Barfoed's reagent and test solution were added. Heated for 1-2 min, in boiling water bath and cooled. Red precipitates were observed.

4.2.2 Tests for Hexose Sugars

Cobalt-chloride test: 3 ml of test solution was mixed with 2ml cobalt chloride, boiled and cooled. Added FeCl₃ drops on NaOH solution. Solution observed for greenish blue (glucose), purplish (Fructose) or upper layer greenish blue and lower layer purplish (Mixture of glucose and fructose).

4.2.3 Tests for Non-Reducing Sugars

a) Test solution does not give response to Fehling's and Benedict's test.

b) Tannic acid test for starch: With 20% tannic acid, test solution was observed for precipitate.

4.3. Tests for Proteins

4.3.1. Biuret test (General test): Took 3 ml of T.S. added 4% NaOH and few drops of 1% CuSO₄ solution observed for violet or pink color.

4.3.2. Million's test (for proteins): Mixed 3 ml of T.S. with 5 ml Million's reagent, white precipitate. Precipitate warmed turns brick red or precipitate dissolves giving red colour was observed.

4.3.3. Xanthoprotein test (For protein containing tyrosine or tryptophan): Mixed 3 ml of T.S. with 1 ml concentrated H₂SO₄ observed for white precipitate.

4.3.4. Test for protein containing sulphur: Mixed 5 ml of T.S. with 2 ml 40% NaOH and 2 drops 10% lead acetate solution. Solution was boiled it turned black or brownish due to PbS formation was observed.

4.3.5. Precipitation test: The test solution gave white colloidal precipitate with following reagents:

- absolute alcohol
- 5% HgCl₂ solution
- 5% CuSO₄ solution
- 5% lead acetate
- 5% ammonium sulphate

4.4. Tests for Steroid

4.4.1. Salkowski Reaction: Took 2 ml of extract and 2 ml chloroform and 2 ml concentrated H₂SO₄ was added. Shacked well, whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence was observed.

4.4.2. Liebermann-Burchard Reaction: Mixed 2ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops concentration H₂SO₄ from the side of test tube observed for first red, then blue and finally green colour.

4.4.3 Libermann's reaction: Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H₂SO₄ observed for blue colour.

4.5 Tests for Amino Acids

4.5.1 Ninhydrin test (General test):- 3 ml T.S. and 3 drops 5% Ninhydrin solution were heated in boiling water bath for 10 min. observed for purple or bluish colour.

4.5.2 Test for Tyrosine: Heated 3 ml T.S. and 3 drops Million's reagent. Solution observed for dark red colour.

4.5.3 Test for tryptophan: Take 3 ml T.S. added few drops glyco-oxalic acid and concentrated H₂SO₄ observed for reddish violet ring at junction of the two layers.

4.6. Tests for Glycosides

4.6.1 Tests for Cardiac Glycosides

4.6.1.1 Baljet's test:- A test solution observed for yellow to orange colour with sodium picrate.

4.6.1.2 Legal's test (For cardenoloids):- Took aqueous or alcoholic test solution, added 1 ml pyridine and 1 ml sodium nitroprusside observed for pink to red colour.

4.6.1.3 Test for deoxysugars (Kellar Killani test):- Took 2 ml extract added glacial acetic acid, one drop of 5% FeCl₃ and concentrated H₂SO₄ observed for reddish brown colour at junction of the two liquid and upper layers bluish green.

4.6.1.4 Libermann's test (For bufadenolids):- Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H₂SO₄ observed for blue colour.

4.6.2. Tests for Saponin Glycosides

4.6.2.1 Foam test: The drug extract or dry powder was shake vigorously with water. Persistent foam was observed.

4.6.2.2 Haemolytic test: Added test solution to one drop of blood placed on glass slide. Hemolytic zone whether appeared was observed.

4.6.3. Tests for Coumarin Glycosides

Test solution when made alkaline, observed for blue or green fluorescence.

4.7. Tests for Flavonoids

4.7.1 Shinoda test: - To dried powder or extract, added 5 ml 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings. Pink colour was observed.

4.7.2 To small quantity of residue, added lead acetate solution observed for Yellow coloured Precipitate.

4.7.3 Addition of increasing amount of sodium hydroxide to the residue whether showed Yellow coloration, which was decolorized after addition of acid, was observed.

4.7.4 Ferric chloride test: - Test solution, added few drops of ferric chloride solution observed for intense green colour.

4.8. Tests for Alkaloids

4.8.1 Dragendorff's test: Took 2-3 ml test solution added few drops Dragendorff's reagent observed for orange brown precipitate.

4.8.2 Mayer's test:- Took 2-3 ml test solution with few drops Mayer's reagent observed for precipitate.

4.8.3 Hager's test:- Took 2-3 ml test solution with Hagers reagent observed for yellow precipitate.

4.8.4 Wagner's test:- Took 2-3 ml test solution with few drops of Wagner's reagent observed reddish brown precipitate.^[17]

4.9. Tests for Tannins and Phenolic Compounds

Took 2-3 ml test solution, added few drops of whether showed following was observed

a) **5% FeCl₃ solution:** Deep blue-black coloured.

b) **Lead acetate solution:** White precipitate.

c) **Gelatin solution:** White precipitate.

d) **Bromine water:** Decoloration of bromine water.

e) **Acetic acid solution:** Red colour solution.

f) **Potassium dichromate:** Red precipitate.

g) **Dilute iodine solution:** Transient red colour.

h) **Dilute HNO₃:-** Reddish to yellow colour.

5. RESULT

1. Drug - *Berberis Aristata*

S. NO.	Test	Positive & negative
A-	Test for carbohydrate	
	Molish Test(General Test)	Positive
I	For reducing Sugars	
a.	Fehlingh Test	Positive
b.	Bendicts Test	Positive
ii	Test for Monosaccharides	
a.	Barfoed test	Negative
b.	Test for hexose sugars	-
iii	Test non reducing sugars	
a.	Test solution does not gavetave response to fehlingh & venedicts Test	-
b.	Tannic Acid Test for Starch:	Negative
	With 20% Tannic Acid test solution was observe precipitate	
B.	Test for proteins	
a.	Biuret test (General Test)	Negative
b.	Milliions Test (for Portions)	Negative
c.	Xanthoprotein Test (for protines contining, Try Ptophan)	Negative
d.	Test for Protines containing sulphur	Negative
e.	Precipitation Test	
i)	Absolute-Alcohol	Negative
ii)	5% Hgcl2 Solution	Negative

iii)	5% CuSO_4 Solution	Negative
iv)	5% Lead Acetate	Negative
v)	5% Ammonium Sulphate	Negative
C.	Test for steroid	
a.	Salkowski Reaction	Positive
b.	Liebermann-Burchard Reaction	-
c.	Liebermann's reaction	-
D	Tests for Amino Acids	
a.	Ninhydrin test (General test):	Negative
b.	Test for Tyrosine	Negative
c.	Test for tryptophan	-
E	Tests for Glycosides:	
1.	Tests for Cardiac Glycosides	
a.	Baljet's test	Positive
b.	Legal's test (For cardenoloids)	Negative
c.	Test for deoxysugars (Kellar Killani test)	Negative
d.	Liebermann's test (For bufadenolids)	-
2.	Tests for Saponin Glycosides:-	
a.	Foam test	Positive
b.	Haemolytic test	-
c.	Tests for Coumarin Glycosides:-	Negative
F	Tests for Flavonoids	
a.	Shinoda test	Positive
b.	To small quantity of residue, added lead acetate solution observed for Yellow coloured precipitate.	Positive
c.	Addition of increasing amount of sodium hydroxide to the residue whether showed yellow colouration, which was decolourised after addition of acid was observed.	Positive
d.	Ferric chloride test	Negative
G.	Tests for Alkaloids	
a.	Dragendroff's test:	Positive
b.	Mayer's test	Negative
c.	Hager's test	Positive
d.	Wagner's test	Negative
H.	Tests for Tannins and Phenolic Compounds	
a.	5% FeCl_3 solution	Negative
b.	Lead acetate solution	Positive
c.	Gelatin solution	-
d.	Bromine water	-
e.	Acetic acid solution	Negative
f.	Potassium dichromate	Positive
g.	Dilute iodine solution	Positive
h.	Dilute HNO_3	Positive

2. Drug - *Picorrhiza Kurroa*

S. No.	Test	Positive & Negative
A-	Test for carbohydrate	
I	Molish Test (General Test)	Negative
II	For reducing Sugars	
a.	Fehling's Test	Positive
b.	Benedict's Test	Positive
I	Test for Monosaccharides	
1.	Barfoed's test	Negative
2.	Test for hexose sugars	-
II	Test non reducing sugars	
a.	Test solution does not give response to Fehling's & Benedict's Test	-
b.	Tannic Acid Test for Starch:	
c.	With 20% Tannic Acid test solution was observed precipitate	Negative
B.	Test for proteins	

a.	Biuret test (General Test)	Negative
b.	Milliions Test (for Portions)	Negative
c.	Xanthoprotein Test (for protines contining, Try Ptophan)	Negative
d.	Test for Protines containing sulphur	Negative
C.	Precipitation Test	
i)	Absolute-Alcohol	Negative
ii)	5% Hgcl ₂ Solution	Negative
iii)	5% Cuso ₄ Solution	Negative
iv)	5% Lead Acetate	Negative
v)	5% Ammonium Sulphate	Negative
D.	Test for steroid	
a.	Salkowski Reaction	Positive
b.	Liebermann-Burchard Reaction	-
c.	Liebermann's reaction	-
E.	Tests for Amino Acids	
a.	Ninhydrin test (General test):	Negative
b.	Test for Tyrosine	Negative
c.	Test for tryptophan	-
F.	Tests for Glycosides:	
1.	Tests for Cardiac Glycosides	
a.	Baljet's test	Positive
b.	Legal's test (For cardenoloids)	Negative
c.	Test for deoxysugars (Kellar Killani test)	Negative
d.	Liebermann's test (For bufadenolids)	-
2.	Tests for Saponin Glycosides:-	
a.	Foam test	Negative
b.	Haemolytic test	-
c.	Tests for Coumarin Glycosides:-	Negative Negative
G.	Tests for Flavonoids	
a.	Shinoda test	Negative
b.	To small quantity of residue, added lead acetate solution observed for Yellow coloured precipitate.	Positive
c.	Addition of increasing amount of sodium hydroxide to the residue whether showed yellow colouration, which was decolourised after addition of acid was observed.	Positive
d.	Ferric chloride test	Negative
H.	Tests for Alkaloids	
a.	Dragendroff's test:	Negative
b.	Mayer's test	Negative
c.	Hager's test	Negative
d.	Wagner's test	Negative
I.	Tests for Tannins and Phenolic Compounds	
a.	5% FeCl ₃ solution	Negative
b.	Lead acetate solution	Negative
c.	Gelatin solution	-
d.	Bromine water	-
e.	Acetic acid solution	Negative
f.	Potassium dichromate	Negative
g.	Dilute iodine solution	Negative
h.	Dilute HNO ₃	Negative

Photochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are carbohydrates, flavonoids, alkaloids, glycosides, steroids are present in *B.aristata* and *Picorrhiza kurroa* may carbohydrate, steroid, cardiac glycoside and flavonids

are present. The most active extracts could be subjected for further pharmacological evaluation by isolation of the therapeutic antimicrobials and further research on this plant can specify its pharmaceutical application.

CONCLUSION

Preliminary pharmacognostical standardization studies of the *Berberis* and *Picrorhiza Kurroa* other physical values and parameters will help to identify the species of plant. The most active extracts could be subjected for further pharmacological evaluation by isolation of the therapeutic antimicrobials and further research on this plant can specify its pharmaceutical application.

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