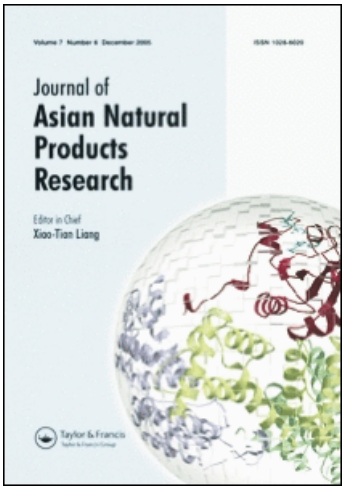

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Colorimetric estimation of total iridoid content of *Picrorhiza kurrooa*

Pushpa Narayanan; K. G. Akamanchi

Online publication date: 12 May 2010

To cite this Article Narayanan, Pushpa and Akamanchi, K. G.(2003) 'Colorimetric estimation of total iridoid content of *Picrorhiza kurrooa*', Journal of Asian Natural Products Research, 5: 2, 105 — 111
To link to this Article: DOI: 10.1080/1028602021000054955
URL: http://dx.doi.org/10.1080/1028602021000054955

Journal of Asian Natural Products Research, 2003 VOL. 5 (2), pp. 105–111

COLORIMETRIC ESTIMATION OF TOTAL IRIDOID CONTENT OF *PICRORHIZA KURROOA*

PUSHPA NARAYANAN and K.G. AKAMANCHI*

University Department of Chemical Technology, Matunga, Mumbai 400019, India

(Received 1 July 2002; Revised 12 July 2002; In final form 29 July 2002)

A colorimetric method has been developed for the selective analysis of the total iridoid content of the rhizomes of *Picrorhiza kurrooa* in terms of catalpol. The method of analysis is based on the reaction between iridoid compounds and primary amine. The iridoid glycosides present in *P. kurrooa* are mainly the esters of catalpol, and can be easily converted into catalpol by saponification. Catalpol thus obtained by hydrolysis is treated with glycine in acidic medium to give a purple color with a maximum absorption of 542 nm. The method was validated as per the ICH guidelines for linearity, accuracy and precision. Several other rhizome samples of the plant were also assayed using this method. The method developed is precise, sensitive, reproducible and easy to perform and can be used for the standardization of crude drug.

Keywords: *Picrorhiza kurrooa*; Iridoids; Colorimetry; Glycine; Catalpol

INTRODUCTION

Picrorhiza kurrooa Royle ex Benth (Scrophulariaceae) is an erect perennial herb about 15 to 25 cm high, growing in the Himalayas from Kashmir to Sikkim at an altitude of 9000–15,000 ft. The common trade and vernacular name of the herb is 'kutki'. The root has a bitter sharp taste and is a valued drug for liver complaints and biliousness. In India it is utilized for the treatment of jaundice, indigestion, common fever, acute viral hepatitis and bronchial asthma [1]. Pharmacological studies have revealed hepatoprotection [2], antiinflammation [3], immunostimulation [4] and free radical scavenging activities [5]. Iridoid glycosides as shown in Fig. 1 are the major chemical constituents and phenyl glycosides, *viz*. androsin, a number of cucurbitacin glycosides and 4-hydroxy-3-methoxyacetophenones are the minor chemical constituents of the rhizomes [6]. There are several HPLC methods [7–9] in the literature for estimation of picroside I and kutkoside which is part of the formulation Picroliv.

Present work is aimed towards the estimation of the total iridoid content of *P. kurrooa*, which could be a simple way of standardization of the crude drug. Iridoids present in the *P. kurrooa* are mainly the esters of catalpol [10]. The iridoids can be easily converted into catalpol by saponification (Fig. 2). Therefore one can develop a method to quantify iridoids in terms of catalpol, which can be isolated from the plant.

*Corresponding author. Tel.: +91-22-4145616. Ext. 422. E-mail: kga@pharma.udct.ernet.in

ISSN 1028-6020 print/ISSN 1477-2213 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/1028602021000054955

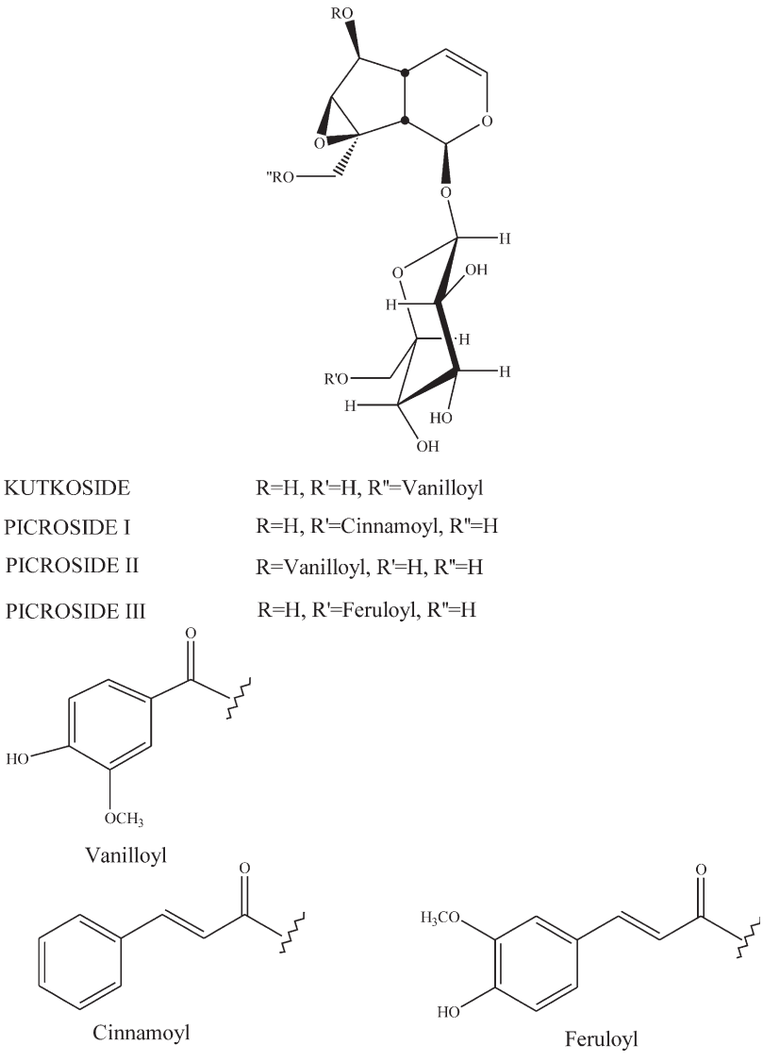

FIGURE 1 Iridoid glycosides of *P. kurrooa*.

RESULTS AND DISCUSSION

The present work has been developed on the basis of the reaction between hydrolyzed products of the iridoid glycosides and glycine, which was reported first in 1960 [11]. It is known that the iridoid aglycones react with primary amines to form colored substances. Such colors include black, dark purple, purple, light purple, blue–purple, blue, green, yellow–green, gold, orange, brown, dark brown, light brown, gray, *etc*. These substances can be used for coloring foods, medicaments or cosmetics [12]. The iridoids can react with compounds contained in hair and, having functional groups such as amino or hydroxyl in the same state as those in hair, produce colored substances of various tone of color. Accordingly, the glycosides and aglycones can be used to give a wide variety of colors to hair [12].

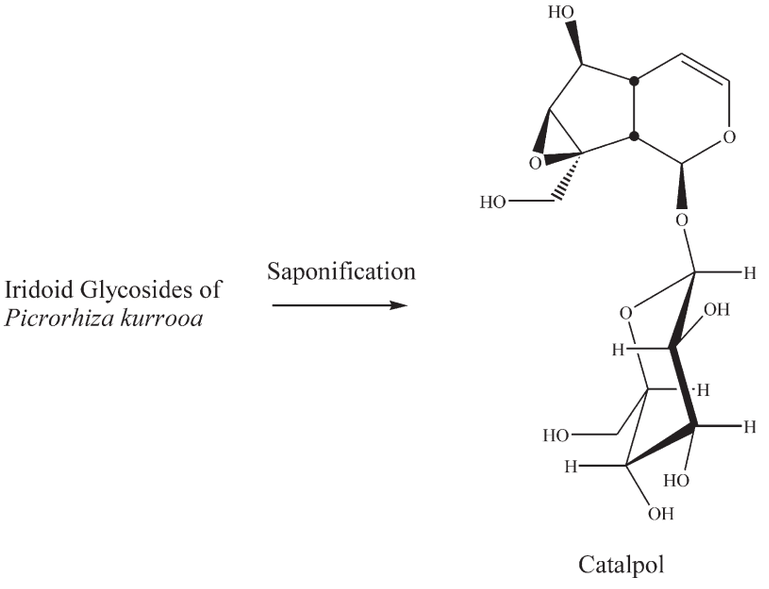

FIGURE 2 Formation of catalpol.

Iridoid compounds when present as glycosides can be hydrolyzed to yield the aglycone and then the aglycone reacts with a substance possessing a primary amino group under acidic condition to produce a red-coloring composite with a maximum absorption at 520–540 nm. It is also reported that the hue of the coloring composite can be controlled by selecting the kinds of primary amines and iridoid compounds, and by changing the conditions of the reaction. Iridoid aglycone is obtained by application of microorganism or enzyme or chemical methods [13]. Based on this literature the color reaction of catalpol with the amino acid glycine was developed; 0.1 M sulfuric acid was used for hydrolysis of the glycoside. The color development occurs at room temperature but to accelerate the reaction it is heated in boiling water bath (100°C). The reaction was studied over a period of one hour. It was found that a stable color was developed within 20 min with no further change in absorbance values. The solution is then filtered. A purple colored solution is obtained which shows a λ_{max} 542 nm in the visible region.

The absorbance pattern of treated catalpol and that of the treated extract are shown in Fig. 3, both showing a λ_{max} of 542 nm.

The aglycone obtained by hydrolysis of iridoid glycosides is very reactive. It reacts with the primary amino group to form a nitrogen-containing monoterpene derivative having a pyridine skeleton wherein the oxygen atom in the aglycone is replaced with a nitrogen atom of the primary amino group. These compounds give color to the solution. This color complex was found to be an intractable mixture of high-molecular-weight polymers on the basis of its chromatographic behavior, unanalyzable ^{13}C NMR spectrum and by molecular weight measurements [14,15].

METHOD OF ANALYSIS

A colorimetric method of analysis was developed on the basis of the reaction of the amino acid glycine with the aglycone generated *in situ*. The solution showed a λ_{max} at

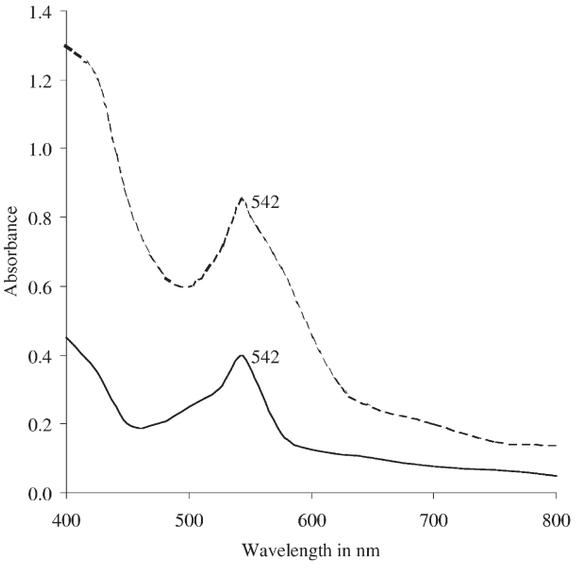

FIGURE 3 Absorbance spectra of standard catalpol treated with amino acid (—) and extract treated with amino acid (- - -).

542 nm. The method was validated as per the ICH (International Conference of Harmonization) guidelines.

Validation of Method

The method was validated for linearity, accuracy and precision.

Linearity: Stock solutions were diluted to prepare $0-100\,\mu g\,ml^{-1}$ solution and studies were performed for 3 days in succession. Table I gives the regression equation and correlation coefficients for the linearity curve.

Accuracy: The solutions were spiked with 25%, 50% and 100% of pure sample and the percent recovery data were obtained. Table II gives the average recovery data. Standard addition and recovery studies were also performed with the extract. A fixed amount of the extract was spiked with 25%, 50% and 100% of the standard and the percent recovery was calculated and found to be 99%, which complies with the ICH guidelines.

Precision: Precision is the degree of reproducibility, or of repeatability, of an analytical method under normal operating conditions. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation of a series of measurements. Four different readings were taken for the same concentration of sample and the RSD was found to be 0.025. Multiple solutions were prepared and the RSD was calculated to be 0.049.

TABLE I Linearity of the method at λ_{max} 542 nm*

Range (μg)	*Equation of line*	*Correlation coefficient*
0–100	$Y = 0.0060x$	0.9727
0–100	$Y = 0.0041x$	0.9849
0–100	$Y = 0.0047x$	0.9932

* All values as mean ± S.d. $n = 3$.

TABLE II Recovery data at λ_{max} 542 nm*

Actual concentration (μg)	*Calculated concentration* (μg)	*Percent recovery*
80	80.7 ± 0.03	100.87
100	99.6 ± 0.06	99.60
150	150.9 ± 0.05	100.60

* All values as mean ± S.d. $n = 3$.

Stability: The stability of the color was studied for 24 h. It was found to be stable for that period. Stability data are given in Table III.

After the method was developed with the standard compound it was applied to the saponified extract to quantify the total iridoid content as given in Table IV. The average total iridoid content was found to be 3.53%.

Three separate collections of the rhizomes of the plant were also analyzed by this method and the results are tabulated in Table V.

In conclusion this method is simple, sensitive, reproducible and easy to perform. It can be used for the quantification of total iridoids in *Picrorhiza kurrooa* and for standardization of the crude drug. This method can also be used for the comparison of total iridoid contents from various sources.

EXPERIMENTAL

General Procedures

Catalpol used for the standard curve development was purified from *P. kurrooa* root extract and authenticated by NMR spectroscopy and by thin-layer chromatographic (TLC)

TABLE III Stability data

	Absorbance λ_{max} 542 nm				
Sample concentration (μg ml^{-1})	0 h	2 h	4 h	8 h	24 h
50	0.392	0.400	0.391	0.411	0.412
80	0.495	0.499	0.500	0.496	0.511

TABLE IV Estimation of total iridoid content

Solution	*Absorbance λ_{max} 542 nm*	*Iridoid content* (%)
Standard (50 μg ml^{-1})	0.395	–
Extract 1	0.636	4.025
Extract 2	0.495	3.100
Extract 3	0.516	3.260
Extract 4	0.595	3.765

TABLE V Total iridoid content in terms of catalpol of different plant samples

Sample source	*Iridoid content in terms of catalpol* (%)	*Catalpol isolated* (%)
Jalgoan fields, Maharashtra	1.0–1.5	1.0
Sami labs, Bangalore	2.8–3.1	3.0
All India Kirana Stores (local supermarket), Mumbai	1.7–2.0	1.5

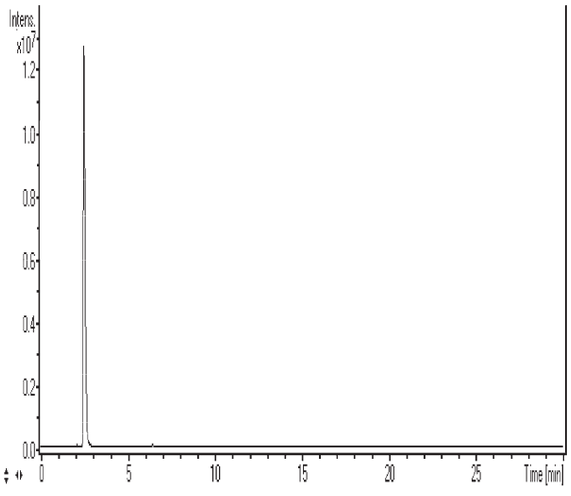

FIGURE 4 HPLC of catalpol.

comparison with an authentic sample [16,17]. Fig. 4 gives the HPLC purity of the compound, catalpol. HPLC was performed using a Jasco HPLC, column C-18, 4.6 × 250 mm, eluant methanol/water (60:40), detected at λ_{max} 220 nm. A Cecil CE2021 spectrometer with 1 cm path-length quartz cells was used for absorbance measurements.

Rhizomes of *P. kurrooa* were purchased from a local market and were standardized as per the Indian Herbal Pharmacopoeial standards. All reagents and solvents were of analytical reagent grade, procured from Rankem fine chemicals, and distilled water was used to prepare the reagent solutions.

Solutions

Standard Catalpol Solution (1000 $\mu g\, ml^{-1}$): Catalpol (0.1 g) was dissolved in 100 ml of distilled water to obtain a 1000 $\mu g\, ml^{-1}$ solution.

Glycine Solution 10%: 10 g of glycine was diluted to 100 ml with distilled water.

Sulfuric Acid Solution (0.1 M): This was prepared by diluting 5.4 ml (1.84 $g\, ml^{-1}$) of concentrated H_2SO_4 to 1000 ml.

Procedure for Calibration Graph

In each series of 10 ml calibrated flasks was placed 0–1 ml of the 1000 $\mu g\, ml^{-1}$ catalpol solution and the aqueous volume was adjusted to 3 ml by addition of the requisite amount of distilled water. Subsequently 1 ml of 10% glycine solution and 1 ml of 0.1 M sulfuric acid were added and the mixture was allowed to stand for 20 min in a boiling water bath. The overall aqueous volume was made up to 10 ml. The reagent blank was prepared simultaneously and its absorbance measured at λ_{max} 542 nm.

Preparation of Extract

P. kurrooa, the drug of which total iridoid content was to be estimated, was dried to constant weight and powdered. The extract, prepared by refluxing 1 g of the drug with 10% of KOH in

distilled water, was filtered and diluted appropriately (100 ml) and then used for analysis. The procedure for analysis was the same as that used for the calibration graph. The solution thus obtained was filtered before recording the readings.

Acknowledgements

Pushpa Narayanan is thankful to the University Grants Commission, New Delhi for financial support.

References

[1] Chopra, R.N. (1958), *Indigenous Drugs of India* (D. N. Dhur & Sons Ltd, Calcutta), p. 182.
[2] Dhawan, B.N. (1995), *Med. Chem. Res.* **5**, 595–605.
[3] del Carmen Recio, M., Maria Ginger, R., Manez, S. and Rios, J.L. (1994), *Planta Med.* **60**, 232–234.
[4] Puri, A., Saxena, R.P., Sumati, Guru, P.Y., Kulshreshtha, D.K., Saxena, K.C. and Dhawan, B.N. (1992), *Planta Med.* **58**, 528–532.
[5] Chander, R., Kapoor, N.K. and Dhawan, B.N. (1992), *Biochem. Pharmacol.* **44**, 180–183.
[6] *Indian Herbal Pharmacopoeia* (1998) (Indian Drug Manufacturers Association, Worli, Mumbai-18 and Regional Research Laboratory, Jammu), Vol. I, p. 106.
[7] Stuppner, H. and Wagner, H. (1992), *Sci. Pharm.* **60**, 73–85.
[8] Dwivedi, A.K., Chaudhry, M. and Seth, K.K. (1989), *Ind. J. Pharm. Sci.* **51**, 274–275.
[9] Dwivedi, A.K., Kulkarni, D. and Singh, S. (1997), *J. Chromatogr. Biomed. Sci. Appl.* **698**, 317–320.
[10] Singh, B. and Rastogi, R.P. (1972), *Ind. J. Chem.* **10**, 29–31.
[11] Djerassi, C., Gray, J.D. and Kinci, F.A. (1960), *J. Org. Chem.* **25**, 2164–2167.
[12] Komai, K., Harima, S. (1992), *US Patent* 5, 078,750.
[13] Touyama, R., Inoue, H., Shingu, T., Takeda, Y., Ikumoto, T., Okuyama, H. and Yamamoto, O. (1981), *US Patent* 4, 247, 698.
[14] Touyama, R., Inoue, K., Takeda, Y., Yatsuzuka, M., Ikumoto, T., Moritome, N., Shingu, T., Yokoi, T. and Inouye, H. (1994), *Chem. Pharm. Bull.* **42**, 668–673.
[15] Touyama, R., Inoue, K., Takeda, Y., Yatsuzuka, M., Ikumoto, T., Moritome, N., Shingu, T., Yokoi, T. and Inouye, H. (1994), *Chem. Pharm. Bull.* **42**, 1571–1578.
[16] Weinges, K., von der Eltz, H., Jaggy, H. (1983), *US Patent* 4401825.
[17] Chaudhuri, K.K., Afifi-Yazar, F.U., Sticher, O. and Winkler, T. (1980), *Tetrahedron* **36**, 2317–2326.